

Is science really a pack of lies?

The past few years have seen a spate of allegations of fraud in science, with the result that the integrity of the research enterprise has been questioned.

WITHIN THE scholarly community, it is widely and easily accepted that most other kinds of professional people cheat. Businessmen, it is well-understood, tell the best tales that can be told of their new products for fear that, otherwise, the products might not be saleable. Politicians are cynically supposed to be prone to a more serious temptation — that of knowing that if they fail to pretend that their latest package of nostrums will succeed in curing all the world's ills, they will not be re-elected in democratic societies. And people who work for large organizations, private (say a multinational commercial enterprise) or public (say a government), are excused from the strict requirement that they should always tell the truth, or tell all of it, on the grounds that too much honesty might entail losing an irreplaceable salary.

Among scientists, there has therefore grown up the belief that most other kinds of professional people are likely to be motivated by self-interest, particularly on those occasions when their professional advice would be most valuable. But, the argument goes, by definition the practice of science must be different, for science cannot make progress but can only go backwards if it defaults on the principle that the only data that matter are those objectively acquired. So what is to be made of the rash of allegations, in the past few years and weeks, that some ingredients of the scientific record are not merely false (which is forgivable) but fraudulent (which implies forethought and perhaps malice)?

Cloning results

These questions are provoked by the sad case of how the University of Geneva has found it necessary to set up an external committee to inquire into the validity of data on mammalian cloning reported in the past several years by Dr Karl Illmensee. Recently, it will be recalled, the Free University of Berlin was compelled by events to set up a similar procedure in respect of another scientist. In each case, the university has been forced, with understandable and proper reluctance, to follow these unpalatable procedures so as to defend itself from charges that its scholars are corrupt and that its own integrity as a scholarly institution is therefore suspect.

The most trivial but still important thing to say about these occurrences is that they are a confounded nuisance. People working in a field in which published data are alleged to be false will find themselves propelled not to creative work but to the repetition of experiments already carried out. Fields of inquiry made muddy in such a way may most simply be dealt with by neglect — young people would be forgiven if they chose to work in some other field instead. And how should journals stuck with suspect data set about informing future archival readers that something published many years ago should not be taken at its face value?

The more magisterial answer to the charge that even the scientific profession may be open to the temptation of fraud is essentially that given by a panel of distinguished witnesses, the late Dr Philip Handler among them, to Congressman Albert Gore's subcommittee of the US House of Representatives two years ago — "Sure, there are a few cheats, but the penalties of being found out are so great, as is the risk, that the few cases that come to light and the fuss they cause are a proof that these must be isolated circumstances — and a sign of how vigilant people's colleagues have become". Extenuating circumstances were also

on that occasion prayed in aid — the pressures that persuade young people that they must make their reputations quickly, usually by publishing in reputable journals, the need to compete for grants and, shockingly, the circumstances that require such a person to act as some notional supervisor's amanuensis while he is occupied with the usual round of academic committees, filling in grant applications or giving evidence to a congressional committee. Others privately (but not, repeat not, for publication) insist that everything was so much simpler when the profession was so much smaller that almost all practitioners could expect to know each other.

The extenuating circumstances are real but do not constitute an excuse. For younger people, the pressure to publish stems from the need to secure a position in research — a PhD is a good start, almost a *sine qua non*, but even the most self-respecting organizations, industrial as well as academic, also look for a sprinkling of published papers ("in the press" is a second best). The requirement is intrinsically illogical, and is most easily met by the handful of outstanding young people who mature early, and who promptly gravitate to the leading research institutions in their fields. But the most serious pressure to publish comes from less distinguished institutions, determined to ape their betters and to enforce as rigorously as they can the most demanding objective criteria that they know of. And the irony is that the dozen or so proven cases of falsification that have cropped up in the past five years have occurred in some of the world's most distinguished research institutions — Cornell, Harvard, Sloan-Kettering, Yale and so on — and have been blamed on people who are acknowledged by their colleagues to have been intellectually outstanding. The pressure to publish may explain much dull literature, but cannot of itself account for fraud.

The indifference of many senior people to what their junior colleagues do in the laboratory is more serious. In at least three of the proven falsifications in the United States, a relatively senior scientist has been in effect a front-man for a junior colleague, acting as a general provider of services and funds and putting his name on an occasional published paper. In both these roles, such people have helped make fraud possible and persuasive — yet it is the junior colleague whose career has been ruined. The natural justice in that arrangement is not easily discerned. And while it would be over-zealous to ask that every author of every scientific paper should be able personally to vouch for every detail of it, there is a point nearer to close knowledge than to near ignorance at which research supervisors and directors should shoulder those actually responsible for a piece of research with the awesome responsibility that full credit carries with it.

Peer review

This principle — letting responsibility lie where credit falls — needs to be even more widely extended. During the rapid growth of the research enterprise in the past three decades, research institutions, universities especially, have slipped into the sloppy habit of substituting for their own judgement of their own achievements the judgement of external assessors as delivered by the appropriate sub-net of the peer-review system. By doing so, they have implicitly agreed that their own corporate judgement of a colleague's work may be partly, or temporarily, suspended if there is evidence that anonymous peers approve.



Nobody can deny the value of the reviewing system as a means of deciding what to do in some practical situation — to publish or not, for example — but a research laboratory jealous of its reputation has to devise less formal, more intimate ways of forming a corporate judgement of the work its people do. The best laboratories and university departments are well-known for their searching mutual questioning. Smaller institutions are less well-placed, but are not those at which things have fallen conspicuously through the cracks. In the long run, it may be in the interests of the scientific enterprise that research people should be more dependent on the judgement of their close colleagues for the funds they can dispose of.

Palliatives such as these would not for certain make accusations of falsification disappear, however. For the unpalatable possibility must remain that even professional scientists may have more in common with other professional people — businessmen and politicians, for example — than they would like to think. Deliberate fraud in an academic setting is undoubtedly rare (and could be made even more so if universities more assiduously cultivated openness) but who could say the same of, say, unthinking or unconscious self-deception? What happens if a person finds some unexpected result in the laboratory, publishes it, wins all kinds of acclaim — and then finds that his result cannot be repeated? To say that he should instantly recant is to ask a lot of flesh and blood.

But does not such tolerance imply that dishonesty can be accepted? Of course not. There is, however, a problem of definition. To be in error is not to be disgraced. Aristotle is not scorned for having foisted a mistaken notion of mechanics on the succeeding millennium. Lord Kelvin's statue in Glasgow has not been smashed because of what he did to make the theory of the aether fashionable in the nineteenth century. But who can be sure that these errors were honest in some absolute sense, and in no whit the products of that mixture of confidence and the determination to win most simply called arrogance? To be sure, it takes extra gall to write down imagined data (and the temptation should be suppressed).

It is foolish, however, to suppose that failings such as these, regrettably common in most fields, should never occur in science. A better hypothesis would be that they should be about as commonplace as elsewhere, from which it follows that there is no way of getting rid of falsification. The best hope is that its consequences can be kept in check. In spite of the seamy record of the past few years, that seems to have been done reasonably. □

Research hopes fade

The promised increase of US spending on research may be illusory.

THE euphoria with which US scientists as recently as February greeted President Reagan's 1984 budget plan, with its proposed 17 per cent increase for research and development, has not survived the spring. Since February, the House of Representatives has decided it wants to add \$1,250 million in the form of a "civilian R&D initiative". Now the Senate is about to consider a proposal to add another \$1,000 million to the research authorizations of five major federal agencies. But in Detroit last weekend, the mood of professional budget-watchers attending the annual meeting of the American Association for the Advancement of Science (AAAS) was one of anxiety rather than opportunity. And this is not as paradoxical as it may seem: there has always been less than meets the eye to the Administration's generosity towards science. The most conspicuous deficiency of the President's spending plan is its disproportionate focus on defence research — forecast in the Administration's plan to consume more than two out of every three federal research and development dollars in 1984. Individual disciplines, too, are treated disproportionately. The bulk of the increases is to go to the physical sciences, mathematics and engineering. The medical, biological and other sciences will receive only a tiny increase or, when the full effects of inflation are computed, significant reductions.

Dr George Keyworth, the President's science adviser, makes no secret of the fact that the Administration wants to direct increases in research spending to those areas it regards as specially important. The science community, for its part, has been willing to accept that a bigger say in the selection of research priorities is part of the price the Administration will demand for a generous increase in overall spending on science. There are, however, two reasons for the new anxieties which clouded the AAAS meeting. First, it is only now becoming clear how little new money will eventually trickle through the defence structure to support basic research in the universities. And second, the nature of the research priorities selected by the Administration has raised questions about the durability of its enthusiasm for science.

Universities used to receiving basic research grants from the Department of Defense (DoD) have been complaining for several months that the money earmarked for basic research is being diverted to applied research. DoD and the Association of American Universities have taken these suspicions seriously enough to start a major investigation. At the AAAS meeting, Professor Robert Park, Washington director of the American Physical Society, drew attention to a potentially more alarming trend: the redefinition of basic research by DoD to enable a growing proportion (nearly half) of its basic research budget to be diverted from unsolicited research grants to a group of "new initiatives". These initiatives, he claimed, were dominated by research in areas such as "the man-machine interface"; low-speed landing and take-off; and spacecraft survivability. If these charges are true, the outlook for the health of basic science in the universities is profoundly discouraging. It is, after all, the basic research component of the defence budget and the Department of Energy's budget for new big machines in physics that make up the bulk of the increase in spending on science under the Administration's plan. That the increase in spending is more apparent than real is a point that might well elude those — including the President — who are pinning their hopes for an economic recovery on the imagined ability of basic research to stimulate high technology and spawn new industries and jobs. The great danger is that the Administration, and some in Congress, expect too great a return from too small an investment in science. When their expectations are frustrated, will the science budget be allowed to revert to its downward trend?

Congress appears to offer one source of comfort. At the very least, it can be expected to correct (but not eliminate) the Administration's disproportionate emphasis on defence in spending plans for 1984. It will also give the biomedical sciences, and the National Institutes of Health, the increases denied them in the budget. But congressional support for science cannot be taken for granted over the longer term or over the whole range of agencies and disciplines. What is clear is that non-defence research and development will face tremendous competition to sustain its share of a shrinking total for non-defence discretionary programmes.

What should the community of scientists do in the face of these dangers? First, it must make it plain to all who will listen that investment in science has to be sustained over many years before it can be expected to yield dramatic economic returns. By saying so, scientists will be accused of being ungrateful for the preferential treatment they have indeed received in the forthcoming budget. But it is far more important to scotch the notion that spending on research can be an immediate economic cure-all than it is to remain on friendly terms with a particular administration. The second challenge is to avoid the temptation to allow the present wave of enthusiasm for science spending to subvert normal procedures for allocating priorities in research. At AAAS over the weekend, several speakers were rightly critical of the unseemly scramble for congressional favour by universities and laboratories competing recently for materials research money and for the right to build the next big machine for high-energy physics. Pork-barrel politics is an effective way for the powerful to get extra money for their favourite projects. In the long run, however, science's claim on Congress is better served by adhering to the rigorous standards of peer review. □

Cloning technique under cloud

Geneva and Bar Harbor labs to check results

FOLLOWING allegations by members of Professor Karl Illmensee's laboratory and an internal inquiry, the University of Geneva has set up an external commission to examine his highly publicized experiments of the past five years. The Jackson Laboratory at Bar Harbor, Maine, is also appointing a committee, to investigate the work carried out by Professor Illmensee during his sabbatical visits there.

Illmensee's work has seldom been out of the news since he took up his present post in the department of animal biology at the University of Geneva in 1978. By experimental manipulation of mouse embryos, he has produced mice containing human or rat genetic material, mice without any genetic contribution from the sperm and also cloned mice. The fact that other laboratories have had difficulties in reproducing some of these experiments has usually been put down to Illmensee's unparalleled technical skill in the microsurgery of embryos.

Illmensee has always performed all the key experiments himself. Now, members of his laboratory have alleged certain irregularities in some of his most recent experiments. Inevitably, the external commission will have to consider not only those allegations but their implications for Illmensee's published claims.

Until the inquiries have been completed, the university will not say exactly what the allegations against Illmensee

amount to. It is, however, ominous that they have come from members of his own laboratory and that, after its own internal investigation, the University of Geneva has felt it necessary to mount an independent inquiry. Earlier this week, the membership of the external commission had not been finalized but, said Marcel Guenin, vice-rector of the university, the commission should start its work soon, will take as long as it needs and will publish its final report.

As to the results of the internal inquiry carried out by the dean of the Faculty of Science, Guenin would say only that it revealed "no evidence of systematic fraud" but had shown that the "standard rules of keeping experimental protocols had not been followed by Illmensee in 1982". Illmensee, he said, had admitted as much.

The Jackson Laboratory has set up its own inquiry, under Dr Dorothea Bennett of the Memorial Sloan-Kettering Cancer Center, because some of Illmensee's work was carried out there, in conjunction with Dr Peter Hoppe. Illmensee spent a few months at the Jackson Laboratory before taking up his post in Geneva and has spent two brief sabbaticals there since. Hoppe has also spent a sabbatical year in Geneva.

From that collaboration came the famous clutch of three cloned mice that graced the cover of *Cell* in January 1981. The mice were said to have developed from fertilized eggs which had had their own nuclei replaced by those of an embryonic

mouse cell. Other laboratories, particularly that of Davor Soltar at the Wistar Institute, have failed to get eggs of that kind to develop beyond the blastocyst stage.

Nor has anyone succeeded fully in reproducing the Hoppe and Illmensee work published in the December 1977 *Proceedings of the National Academy of Sciences*, which reported the birth of seven "uniparental" mice. These started life as a fertilized egg from which the male chromosomes had been removed and the female chromosomes artificially doubled. Similar eggs produced in other laboratories have never developed past mid-gestation.

Several people with first or second hand experience of trying to reproduce the techniques, spoke last week of the great skills involved. One emphasized that related techniques, pioneered in the laboratory of Beatrice Mintz at the Fox Chase Institute for Cancer Research, Philadelphia in the mid-1970s, while Illmensee was there, had initially been very hard to reproduce but were now standard. Another still saw no reason why the techniques should not work.

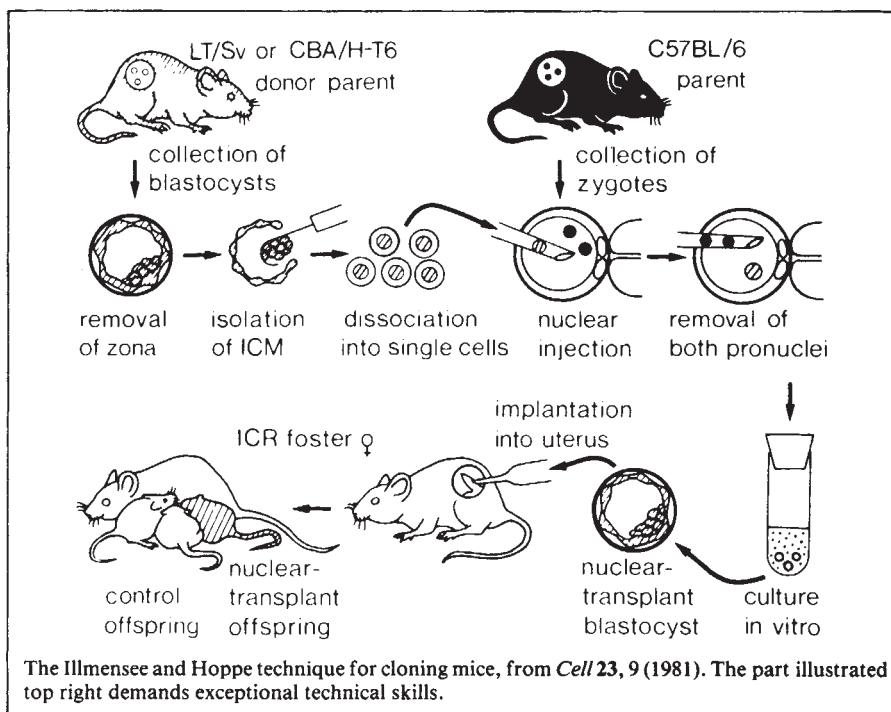
Last week Peter Hoppe had no comment and Karl Illmensee, a Swiss national with a 1971 PhD from the University of Munich, was said to be on holiday. **Peter Newmark**

Pressure to raise US science budget

Washington

A SERIES of amendments designed to add \$1,000 million to President Reagan's proposed science budget for fiscal year 1984 has been drawn up by a group of senators and will be inserted into the budgets of six agencies as their spending plans move through the Senate authorizing procedures in the next few weeks. The amendments would add \$535 million to the National Institutes of Health, \$187 million to the National Science Foundation, \$96 million to the Department of Energy, \$66 million to the National Aeronautics and Space Administration, \$95 million to the Department of Agriculture and £200 million to the Department of Defense.

The spending package was devised by two Missouri senators, Democrat Thomas Eagleton and Republican John Danforth, after detailed talks with the Association of American Universities, which represents the 50 leading research institutions in the United States. In a letter to colleagues, the two senators maintain that the 18 per cent research and development increase proposed by the White House is unevenly distributed. Areas which were given too little attention, they say, include the life sciences and training support of the National Institutes of Health; support for science and engineering postgraduate students; undergraduate science education and the rehabilitation of ageing instruments and laboratories. **Peter David**



French higher education

Assembly begins long wrangle

THE argument about the French Government's hotly disputed bill to reorganize higher education, which occupied the National Assembly for most of last week, will continue at least until October. Despite the pleas of the Minister of Education, M. Alain Savary, that the bill should be dealt with as a matter of urgency, so that its provisions can be enacted in time for the beginning of the academic year in 1984, the French Senate is not expected to finish with the bill until the end of November. Meanwhile, last week, students in Paris and Bordeaux were on the streets again, enjoying better weather for demonstrations than had the farmers who were protesting against agricultural imports the previous week.

The assembly debate, which had the virtue of crystallizing the issues that have made the government's plans contentious, was marked by a fierce denunciation of the bill by M. Raymond Barre, Prime Minister in the pre-Mitterrand government. Calling it "pointless and dangerous", he complained that the bill was an attack on the independence of the universities when the need was for greater autonomy, and that, by contrast, it failed to specify necessary reforms such as that of the *baccalauréat* school-leaving examinations.

M. Barre said that the proposals would create a ponderous, inflexible and uniform system of higher education, offering excessive power to the unions, and that the government had undermined academic freedom by "humiliating" university teachers with its plans to regulate from the centre the transition of students from one phase of higher education to another. Attacking the proposal that the *grandes écoles* (which now operate their own selection system) should be brought within the general orbit of the university system, M. Barre accused the government of a spiteful distaste for the notion of an elite "without which a democracy cannot survive".

Meanwhile, a series of government amendments has defused some of the major issues of the past few weeks. The government proposes to strengthen the representation of teachers and researchers on the proposed university councils, and plans to embody in the bill its compromise with hospital interns last month, when it was agreed that the proposed changes in the pattern of medical education would not take effect until 1987. Even so, it remains to be seen how M. Savary will deal with the spate of amendments put forward both by government supporters (seeking to make the provisions of the bill more explicit) and by the hostile opposition.

M. Savary, whose political future probably depends on his success in carrying the bill more or less intact through the National Assembly, is not at this stage com-

promising on principles. Last week, opening the debate, he insisted that French universities are in no fit state to contribute to economic, social and political development.

On the position of the *grandes écoles*, M. Savary said that the government was not attacking elitism as such, but that the dependence of the *grandes écoles* on public funds implied a "contract with the state" and that it was his intention to see that contract properly negotiated. On the main objective of the bill, Savary also insisted that with two million people in France out of work, it was essential to consider the link between training and employment.

The government's intention that the pro-

motion of students from the first to the second cycles of higher education should be regulated both by examinations and by employment prospects has done as much as any other feature of the bill to bring the students onto the streets, in the process uniting left and right.

This temporary accommodation on the streets will not be mirrored in the assembly, where the government's supporters have been complaining that the present arrangements make higher education the preserve of the middle and professional classes. The parties of the centre-right opposition, however, have promised to use parliamentary procedures to the full so as to delay the bill's passage. Last week, the assembly was still grappling with the first of the 68 clauses of the bill. It will be a long hot summer for M. Savary. □

British election campaign

Nuclear power divides allies

ALL of the major political parties contesting next week's general election seem keen to encourage more selective backing for research, although specific proposals vary. However, one issue that sharply distinguishes the parties is that of nuclear power. The issue also appears to divide the two Alliance partners, the Liberal Party and the Social Democratic Party (SDP).

The Liberals reject nuclear power on economic and safety grounds, and would phase out all nuclear power stations. Mr Christopher Phillips of the SDP's policy unit goes even further by questioning the need for extra power stations of any kind; paradoxically, however, the Alliance manifesto makes a promise to develop research and expertise in the area. The Alliance sees "no evidence" of a need for the pressurized water reactor (PWR) proposed for Sizewell by the Central Electricity Generating Board.

Labour also rejects the "Tory PWR", on similar grounds, but acknowledges the contribution that British advanced gas cooled reactors may make; any further developments in nuclear power must, the party says, proceed on the basis of continued examination of costs and safety. The Conservative Party's manifesto, published last week, says the party would press ahead with the development of nuclear power and set up an Energy Efficiency Office to coordinate the government's conservation effort. Labour and the Alliance also both make commitments to increased efforts in the field of energy conservation.

On higher education, the Conservatives want to see a shift towards technology, science and engineering courses, though within the existing budget. The Alliance and Labour both say they would increase access to higher education and encourage the teaching of scientific and technological subjects. Labour would implement in full

the recommendations of the Alvey report (see page 369), according to science spokesman Mr Geoffrey Robinson. Labour would also nationalize Inmos, the government-backed semiconductor manufacturer. But the Conservatives have laid the most emphasis on new technology in their campaign: Mr Kenneth Baker has drawn attention to the successes of the scheme for supplying microcomputers to schools and the government's "new blood" university appointments in information technology. The Conservatives are especially keen to encourage the development of science parks.

However, it is the SDP that offers the most detailed plans for stimulating technology transfer, and it suggests that the Department of Industry should do more in this direction. It wants a Council for Science Policy to oversee the science effort and to link this with a national innovation policy. The SDP makes specific proposals to increase spin-off benefits from defence-related research. Like the Alliance, Labour wants to reduce defence-related research and would redeploy manpower into "socially useful" areas.

These proposals, ambitious as they are, pale into insignificance when compared with those of the Ecology Party. The party is committed to abolishing all things nuclear and challenges society's "obsession with giant technology"; the party would, if by an electoral miracle it found itself in power, ban the use of all toxic substances. A National Income Scheme would ensure that all received a fair wage, whether working or not; those that were working would be encouraged to look for "good work". There is unexpected agreement between the Ecology Party and Mrs Thatcher on a metaphysical plane: both attach great importance to moral (Conservative) or spiritual (Ecology) values.

Tim Beardsley

AIDS

More money promised

Washington

RESPONDING to charges that the government has neglected acquired immune deficiency syndrome (AIDS), a disease that so far has primarily afflicted male homosexuals and drug addicts, the US Public Health Service (PHS) last week declared AIDS its "number one priority". Dr Edward Brandt, the assistant secretary for health, said PHS would spend \$14.5 million on AIDS research and epidemiological investigations this year, almost as much as has been spent on legionnaire's disease in the entire seven years since that disease was discovered.

PHS, which includes the Centers for Disease Control (CDC) and the National Institutes of Health, has felt increasing pressure to step up work on AIDS from homosexual groups, Congress and more recently — amid reports that AIDS may be spread by blood transfusions — haemophiliacs and others dependent on frequent transfusions.

In making the announcement, Brandt specifically denied that AIDS had been neglected, as some have charged, because it strikes mainly homosexuals. Of the 1,450 cases reported since June 1981, 71 per cent have been in male homosexuals and bisexuals.

Several members of Congress, who called the announcement too little too late, are seeking to boost the PHS budget. Senator Lowell Weicker (Republican, Connecticut) was preparing last week to introduce an amendment to the 1983 supplemental budget — funds that would be made available immediately — that would commit \$12 million specifically for AIDS research. And the House of Representatives Energy and Commerce Committee has already reported out a bill sponsored by Representative Henry Waxman (Democrat, California) that would create a \$30 million fund for public health emergencies beginning in fiscal year 1984. Although this bill does not specifically address AIDS, it is understood that AIDS would immediately qualify as an "emergency" under the bill. (Waxman's bill is HR 2713; the counterpart measure in the Senate, filed by Senator Alan Cranston (Democrat, California) is S 1226).

Brandt emphasized last week that there is no cause for panic about AIDS in the general population; indeed, the entire announcement may have been more an effort to assuage worries than a reflection of a genuinely new policy. Apart from using the occasion to announce the award of six research grants for AIDS studies and the Food and Drug Administration's approval of a new heat treatment for blood products (actually of uncertain utility in preventing transmission of AIDS since the infectious agent is unknown), Brandt gave no indication of what would happen as a

result of the "number one priority" designation that is not already happening.

An aide to Senator Cranston said that most of the \$14.5 million would be diverted from other programmes at CDC. Congress appropriated only \$2 million for CDC's AIDS investigation this year, and \$1 million of that was intended to replace funds that had been diverted in past years to AIDS from other areas, mainly venereal

Plant disease

Italy's farmers battle with virus

BUREAUCRATIC indecision hampers the battle of Italian growers and agricultural scientists against an infestation of plum pox virus, a serious disease of the plum and the apricot, in the regions around Turin and Bologna. The disease could do considerable damage to Italian exports.

Plum pox appears to have come in with root stock from endemically infested Yugoslavia, and so far affects only apricots. It might have been halted in Italy this year if only the infected trees had been destroyed before the aphids flew this spring, but the regional authorities that would have to compensate farmers for the loss of their trees have yet to decide on the principle, let alone the amount of compensation, so farmers are understandably somewhat reluctant to destroy their orchards.

In Turin, the Piedmont regional government has, however, taken the small step of agreeing to pay for a student to work two days a week collecting samples from potentially affected trees, so monitoring the spread of the disease. In Bologna, the disease is said to have a strong grip but to be even less well controlled than in Turin.

Dr Maurizio Conti, who leads a group dealing with plum pox at Italy's leading applied plant virology research institute in Turin, is openly critical of the Piedmont authorities. He claims that they are spending their money in the wrong way. Conti's group detected the outbreak, and warned the authorities, as long ago as last August. But still there is no compensation agreement and so no proper control.

This year, in Piedmont, the spread of the disease has been slowed only by the public-mindedness of one grower, whose orchard of 200 or so trees was 60 per cent affected, the worst in the region. He destroyed them all, without promise of compensation. But there are twelve other foci of infection around Turin where the infected trees still stand.

Conti hopes that the outbreak can nevertheless eventually be contained. In laboratory experiments, he has found that aphids transmit the disease only slowly. With the aid of the student provided by Piedmont, and using an enzyme-linked

diseases and hepatitis. Both Cranston and Waxman blame the administration for not requesting more funds.

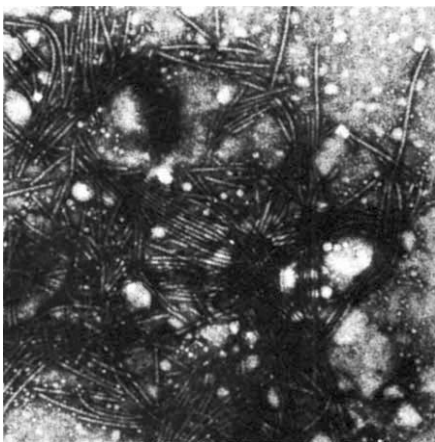
The latest data from CDC, presented last week, underscore the concern about the disease. The fatality rate among those diagnosed two years ago or earlier is 82 per cent; overall, the fatality rate is 38 per cent. Besides male homosexuals, the high risk groups are users of injectable drugs (17 per cent of the reported cases), recent Haitian immigrants to the United States (5 per cent) and haemophiliacs (1 per cent).

Stephen Budiansky

immunoassay system (ELISA) to identify the virus, he is tracking the spread of the virus.

But Conti is running short of the anti-serum that the ELISA technique needs, and has not yet had time to develop a laboratory method of producing it. Moreover, there is a sampling problem. Virus infection is often localized to, say, a single branch of a tree, so that the use of ELISA is confined to confirming a diagnosis on visibly affected branches.

If the disease does establish itself in Italy, the chief consequence will be that the



The offending plum pox virus

export of root stocks will be inhibited. Plum yields will also fall, although not disastrously. In Britain, where one in five of plum orchards is to some degree affected, the fruits of varieties such as the Victoria plum are corrupted while other varieties suffer a loss in yield of 10–20 per cent. In Britain, the disease is contained by close monitoring and grubbing out infected stock.

In Italy, links between farmers and researchers are weaker. Conti says that agricultural ailments tend to be regarded as acts of God. The national plant protection service is mainly concerned with monitoring imported stocks, plainly without success in keeping out plum pox. Conti wants some regular system for demonstrating to farmers the effects of plant diseases so that self-monitoring will be practicable.

Robert Walgate

Atmospheric chemistry

Time to make up for neglect

THE UK Natural Environment Research Council (NERC) is being asked to designate atmospheric chemistry a special topic, which in NERC parlance means that special funds will be allocated for coordinated research grants, not reacting to applications brought in by the mails. The new initiative follows a meeting of scientists from universities, government laboratories and the research councils that highlighted the fragmented state of this discipline in the United Kingdom.

Considerable research effort has, over the past few years, been spent on stratospheric chemistry, primarily as a result of concern over the effect of man-made emissions on the ozone layer. The new initiative reflects recent concern about chemical ignorance of the troposphere (the lowest 10 km or so of the atmosphere), typified most publicly in the problem of acid rain (see also *Nature* 298, 9; 1982).

British efforts in atmospheric chemistry have declined over the past four years. The main government laboratory — the Environmental and Medical Sciences Division at Harwell — has decreased in staff from 8 to 4 over the period as a result of cuts by the Department of the Environment. The number of university groups in the field is in single figures — dwarfed by the United States in proportion to the populations.

This comparison will be unavoidable at a meeting on atmospheric chemistry in Oxford in August where, of the 160 papers scheduled for delivery, 100 or so are from the United States and only 16 from the United Kingdom. Of the latter, only four are from university departments.

The three essential areas of attack highlighted by the meeting were measurements in the troposphere, modelling (for example to understand meteorological effects on chemistry) and laboratory kinetics. The first two aspects of the problem fall under the NERC umbrella, whereas laboratory kinetics is supported by the Science and Engineering Research Council (SERC)'s Science Board.

The need for coordination is obvious and, unsurprisingly, the main proposal that came out of the meeting was for a coordinating committee to be set up with representatives from NERC, SERC, the university community and Harwell (as the most relevant government laboratory).

NERC will decide on this proposal at the beginning of June. Only after such a committee has produced an opinion, and possibly with the help of the Advisory Board for the Research Councils, will NERC decide whether to designate atmospheric chemistry as a special topic and how much to spend on it. **Philip Campbell**

Space Telescope

Counting the astronomical cost

Washington

MANAGEMENT errors by the National Aeronautics and Space Administration (NASA) and a plague of unforeseen technical hurdles have caused massive new cost overruns in the development of the Space Telescope and all but ruled out the possibility of launching it until 1986 or even later. Congressional investigators have told the Appropriations Committee of the House of Representatives that the final cost of building the telescope, originally planned to be \$435 million, is now expected to rise to \$1,000 million.

The investigators' report, by far the most gloomy assessment yet of the troubled programme, pins the blame for soaring costs and delays squarely on the shoulders of NASA and one of its major contractors, the Perkin-Elmer Corporation in Danbury, Connecticut. Perkin-Elmer is building the Optical Telescope Assembly, the heart of the spacecraft and the instrument which

should enable astronomers to observe objects 50 times fainter and 7 times farther away than any existing ground-based device.

Perkin-Elmer has run into major technical difficulties in completing the assembly (see below) but many of them could have been avoided with better management, according to the congressional report. The report says both NASA and the corporation "grossly underestimated" the difficulties likely to arise from the advanced technology being used in the telescope, and NASA consciously decided to bypass the development of "prudent" engineering test models.

One of NASA's biggest errors, the report says, was a decision to limit the number of headquarters staff allocated to the Space Telescope to 90 people, about half the number usually allocated to programmes of similar magnitude and too few to cope with the complexity of the project.

Eventful history

TECHNICAL difficulties have plagued the development of the Space Telescope throughout its life, and congressional investigators have warned the Appropriations Committee that three major hurdles are still threatening to cause long delays, increase costs and impair the efficiency of the telescope when it is eventually launched. The three components causing anxiety are:

- **The Orbital Replacement Unit (ORU) Latches.** Perkin-Elmer is designing 64 latches to perform two crucial functions — aligning and securing the scientific instruments, fine guidance sensors and technical devices within the orbital unit, and enabling space shuttle astronauts to replace the instruments when needed.

In tests at the Marshall Space Flight Center, the latches have tended to chafe and thus prevent an exact fit when they are reclosed. As a result, it is not possible to align the scientific instruments and the fine guidance sensors, which "aim" the telescope, with sufficient accuracy. Perkin-Elmer hopes it can solve the problem by coating the latches with tungsten carbide, but NASA is considering transferring the whole project to Lockheed Missiles and Space Company.

- **The Fine Guidance Sensors.** Four fine guidance sensors, interferometric devices essential to aim the telescope at target objects, are to be built by Perkin-Elmer but the first is only half-finished. NASA doubts whether the corporation is capable of building sensors to meet its own design blueprints, and a review committee has recommended treating the half-completed

sensor as an engineering model for extensive testing. If accepted, the recommendation would add \$100 million to the project and delay a launch until at least April 1986. NASA officials are considering asking Lockheed to take over the project and use Perkin-Elmer as a subcontractor. A partial solution also being considered is to accept some reduction in the pointing accuracy of the telescope.

- **The Primary Mirror.** NASA is asking the scientific community to consider relaxing its specification for the cleanliness of the mirror. After 15 months in a "clean room", dust contamination on the mirror already exceeds the allowable level (no more than a quarter of one per cent of the surface to be contaminated and no dust particles exceeding two-tenths of one millimetre). The dust already accumulated means the mirror can only perform at 70 or 80 per cent of its specified level, raising unresolved questions about how the mirror should be cleaned. The problem of molecular contamination has been warded off by "baking" part of the structure, but at increased cost and with further delays in the timetable. The dust problem is compounded by the fact that the mirror will be in storage for several years.

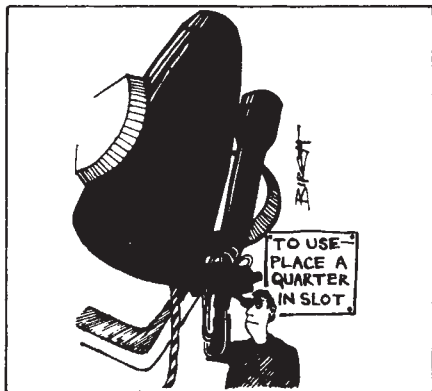
A fourth technical problem looming over the project, but not formally part of it, is the performance of NASA's new sophisticated communication satellite, TDRS (Tracking and Data Relay Satellite System). Communication between the telescope and Earth is to be through TDRS, but the first TDRS, launched in March, tumbled into the wrong orbit and the launch of a second has been delayed until NASA can discover what went wrong. □

The telescope is intended to orbit 320 miles above the Earth for 15 years and is being designed so that it can be serviced and, if necessary, retrieved by astronauts using the space shuttle.

The ceiling of 90 personnel was the result of an agreement between NASA and the Department of Defense, which wanted to limit the number of NASA employees "penetrating" contractors who were also working on classified defence projects. The ceiling was lifted in 1979 but NASA built up to its present level of more than 200 too slowly, the report says. Meanwhile, communications between NASA headquarters and the Marshall Space Flight Center were strained, and Marshall's engineers experienced difficulty in matching their technology with the needs of the science community.

Officials at Marshall admitted to the congressional investigators that they had failed to oversee the work of the Perkin-Elmer Corporation closely enough, having "assumed" that the corporation would be able to work with the same level of NASA supervision as a large aerospace firm. This turned out to be a "grievous and costly error": NASA officials attribute some \$225 million of cost overrun to "deficiencies" in the corporation's handling of the project.

These deficiencies, NASA claims, included unrealistic pricing of the project; an inability to muster a skilled optical work force; a preoccupation with technical excellence regardless of time or cost; "crisis



management" and a reluctance to make management changes until forced by NASA to do so. Early this year, NASA at last took action to retrieve the situation by installing its own 15-member oversight team at Perkin-Elmer, farming some of the corporation's work out to other contractors and creating a six-man "recovery effort" team at headquarters.

The cost overruns on the Space Telescope have been gobbling up a growing share of NASA's budget. James Begg, the agency's administrator, has asked Congress to transfer an extra \$45 million for the programme in fiscal year 1983 by juggling funds in NASA's physics and astronomy programme and plundering space flight operations, the Landsat programme and Spacelab. In fiscal year 1984 an additional sum of between \$40 million

and \$80 million is likely to be switched to the Space Telescope budget with an unknown extra amount required in 1985.

In a letter to the Senate, Beggs says the extra \$45 million for 1983 is needed to solve the technical problems standing in the way of completing the Optical Telescope Assembly, and particularly the fine guidance sensor which enables the telescope to "aim" with sufficient accuracy at distant stars and galaxies. The funds will be used to install additional testing equipment at Perkin-Elmer and purchase more spare parts.

A detailed study of the Perkin-Elmer

contract, provided in the congressional report, says the original \$69.4 million contract awarded in 1977 has increased to \$280 million. Since its inception there have been 141 contract changes, costing about \$173 million. NASA maintains that only \$34 million of these changes were initiated by the government and the rest were a result of the corporation's technical and managerial problems. Despite its poor record, Perkin-Elmer had by last February received more than \$5 million from NASA in fees which are supposed to be based on business management and cost control.

Peter David

Poland's agricultural crisis

Advice coming from all sides

THE Polish Government has presented plans for the rescue of the country's agriculture, and further advice is being offered from all sides. The government's announcement, however, begs a number of questions at the heart of the crisis in Polish agriculture — a crisis closely linked with recent Polish political and social upheavals.

The draft programme for agricultural reform, announced last month by Stanislaw Zieba, Minister of Agriculture and Food Economy, aims to restore self-sufficiency in food "at a level which would make it possible fully to meet the community's needs in accordance with the requirements of a rational diet". Together with an economic rescue operation, the programme includes two variants of long-term action, the final choice of which will be made in 1986. According to Mr Zieba, the government hopes to implement the "more difficult, more ambitious variant", which will include a wide-ranging research programme for land conservation and improvement, water management, mineral fertilizers, high-protein fodders, veterinary medicine and crop protection.

The economic base of the new programme will be "a rational system of prices, agricultural taxes, credits and subsidies, equal for all sectors, and taking into account the needs of all farmers", and the index of success of the plan is to be the *per capita* consumption of meat — with a target, in the most optimistic variant, of 62 kg per person per annum in 1990.

The government's programme, presented to the Sejm (parliament) on 12 May, is not the only recent proposal for the rescue of Polish agriculture. The most important alternatives are the proposal, by the Catholic episcopates of Western Europe and the United States, for the establishment of a special aid fund to finance investment loans to small farmers, and the findings of an agricultural mission to Poland by the Rockefeller Foundation in 1982. These two plans attack the problem from opposite standpoints; the bishops envisage a self-help scheme of loans, the ultimate repayment of which would be administered by the Polish church,

and the building up of an infrastructure of nongovernmental self-managing bodies for purchasing and essential supplies. The Rockefeller scholars conclude that the state sector would have to retain responsibility for purchasing and distribution of farm produce, as well as the provision of essential supplies to the farmers.

Although the Polish Government does not formally support the Rockefeller plan, the latter's acceptance of the current state control of the supply and purchasing system has won for it the off-the-record approval of several Polish "elder statesmen", particularly when contrasted with the bishops' plan which has overtones of the "alternative society" proposed by the underground Solidarity leadership.

The Sejm's proposals, ironically, appear to leave more to the nongovernment sector than the Rockefeller report. There already exists a network of "rural circles" and agricultural self-help groups, which the government, when abolishing the private farmers' "Rural Solidarity" union, pledged itself to maintain and support. Current policy on scientific research puts considerable emphasis on direct contracts, and with the government at present fostering agricultural "self-governance", research contracts between farmers' organizations and institutes seem a possibility.

One major issue, however, is not tackled: the level of meat consumption. During the 1970s meat prices were kept down by massive subsidies as a kind of compensation for the chronic lack of consumer goods. Meat consumption soared and, in order to satisfy the market, high-grade feed grain had to be imported for the livestock producers. According to the Rockefeller scholars, these imports account for some \$7,000 million of Poland's trade deficit. Economists, nutritionists and sociologists are all agreed that meat consumption could well be cut, but the last two attempts to do this led to social unrest and the fall of the Gierek administration. General Jaruzelski's government, in spite of two years of meat rationing, seems unwilling to attempt a long-term reform of eating habits.

Vera Rich

Comecon research

Moscow merely the centre

SOVIET science planners and publicists have taken to emphasizing that much happens outside Moscow, and that the fourteen academies of science of the non-Russian republics exist. And now increasing emphasis is being placed on the role of the republic academies in international collaborative projects within Comecon.

Until recently, Soviet writing on Comecon science stressed mainly the big international agencies and projects such as the Interkosmos space programme. The first hint of a policy shift came with the public announcement in February that the Vega (Venus and Halley's comet) programme was being coordinated from the Kiev observatory of the Ukrainian Academy of Sciences.

Although the reasons for choosing Kiev as the coordinating centre seemed obscure, this might have been a one-off event, with the main emphasis remaining on the Comecon role of the prestigious research institutes in Moscow, Leningrad and Novosibirsk. Last month, however, *Pravda* printed an extensive report on the Minsk Institute of Heat and Mass Exchange, the tone of which suggests that decentralization of Soviet Comecon work is in the ascendant. The article had only marginal topicality and appears to have been written solely as the result of a policy directive.

The Byelorussian Academy of Sciences has a long history of research on heat and mass exchange, and, in the past 20 years, has hosted a number of "All-Union" conferences in this field which included participants from the Comecon bloc. The *Pravda* article, however, stresses the role of the special Comecon centre there — an establishment which, in the diamond jubilee book of the academy in 1979, merited only a two-line mention.

The institute itself has an extensive theoretical programme on matters such as inter-phase heat exchange, the dynamics of combustion and mathematical modelling of diffusion and convection. The *Pravda* reporter, however, while giving credit to the "high authority" of the international centre, seems to have had difficulty in describing the work of an institution more remarkable for a steady programme of research and training than for any spectacular "big" science. He did his best, however, to allude to current Soviet priorities (the food programme and space) by informing his readers that the centre's programme ranges from the drying of food grains to the theory of spacecraft thermal shielding. Computers and lasers, both Byelorussian specialities, rated a mention while proper political emphasis was given to the trainees from two of the less developed Comecon countries (Cuba and Mongolia) and Yugoslavia. **Vera Rich**

US nuclear physics

Argonne picks a fight

Washington

ARGONNE National Laboratory, outside Chicago, has taken to fighting fire with fire in its bid for a new electron accelerator. Having failed to persuade the Department of Energy's Nuclear Science Advisory Committee (NSAC) to forgo an endorsement of the proposal from Argonne's competitor (see *Nature* 28 April, p.743), the laboratory and an assortment of midwestern universities and corporations have taken their case to the political powers that be. Last week, Senator Charles Percy (Republican, Illinois) arranged a meeting with Secretary of Energy Donald Hodel to allow the group to present their claim for the accelerator in a last-ditch attempt to oust the favoured proposal, developed by 23 universities calling themselves the Southeastern Universities Research Association (SURA).

SURA from the start has used all the political leverage it could. Senator John Warner (Republican, Virginia) and others from Virginia in Congress have been particularly active in their support of SURA, a possible magnet for attracting high-technology industry to the region.

Argonne is not shying away from pulling political strings either. Although the recommendations of NSAC, which consists of prominent academic researchers, have usually carried considerable weight, the ultimate decision rests with Hodel. Hence the meeting arranged by Percy, and Argonne's unusual strategy that emerged last week: in a report presented at that meeting (and printed only the day before), Argonne argues that if the SURA design is what NSAC wants, then fine — Argonne will build it, and cheaper, too. According to Argonne's figures, the cost of building and operating the SURA-design machine over its lifetime of 15 years would be \$42 million lower at Argonne than at the proposed SURA site, a vacant laboratory structure in Newport News, Virginia. Argonne claims these savings would accrue from using Argonne's existing support staff, physical facilities and services.

Hodel emerged noncommittal from the closed-door meeting last week. But he did concede a key point in Argonne's strategy: he said that although NSAC is well-suited to make technical judgments, it was not up to NSAC to decide where that design should be constructed.

Percy made much of the alleged monetary savings of building at Argonne and a recommendation last year by the Energy Research Advisory Board that no new national laboratories should be built. He also said that the University of Chicago and the "Big 10" midwestern universities were prepared to offer faculty positions in conjunction with the new accelerator. NSAC, in choosing the SURA proposal,

gave some weight to SURA's commitment of 35 new faculty positions for researchers associated with the facility.

Percy also noted, as did the Argonne report, that the midwest has received a disproportionately small share of federal contracts. "We do not at all begrudge the ship-building efforts in Virginia", Percy said, referring to the considerable naval facilities in Newport News.

An aide to Senator Warner of Virginia said "it was a little late in the game" for Argonne to be presenting a new proposal. And she dismissed Argonne's complaint that it could not have known that so much weight would be given to such criteria as new faculty posts. "Everyone when they're writing a proposal tries to make it innovative", she said, and Argonne had just as much chance as anyone else to put together an attractive package. "It's a little like sour grapes" she said. **Stephen Budiansky**

Trad natural history

THE impact of man on British wildlife is the subject of a permanent exhibition at the British Museum (Natural History) in London, which looks at the general effect that man has had on the land since his first arrival in Britain 20,000 years ago, including the organisms that he has introduced as well as those that he has destroyed. Exhibits are "traditional" museum fare (such as stuffed foxes), not



the more exciting and controversial style of the museum's popular human biology hall. The new exhibition is aimed at the informed amateur and gives an introduction to the techniques of classification and how to apply them. Costing a total of £150,000, the exhibition took almost all of the museum's available cash for 1982, and is intended to last for 15 years. This should give an overall cost of between 1 and 2 pence per visitor, according to its operations manager, Malcolm McBratney. □

Defence-related research

US universities agonize on secrecy

Detroit

THERE are "ominous" signs that the Department of Defense (DoD) is moving towards tighter controls on sensitive scientific information, according to the universities' representative on a DoD committee on export controls. David Wilson, special assistant to the president of the University of California system and co-chairman of the DoD-University Working Group on Export Controls, said at the meeting here of the American Association for the Advancement of Science that the group's efforts to strike a balance between researchers' need to publish and government's need to control sensitive though unclassified information may be undermined by the action of DoD "hard-liners".

University researchers have accused DoD of acting capriciously in several recent clamp-downs on publication or presentation of research results. Most notable was the withdrawal of 150 papers at last year's meeting of the Society of Photo-Optical Instrumentation Engineers in San Diego after DoD officials warned that their presentation might violate export controls. DoD officials noted in particular the presence of foreign nationals at the meeting.

The DoD-University Working Group, part of a larger "forum" set up last year to discuss a wide range of issues of mutual concern, adopted a recommendation in April designed to eliminate such arbitrary actions and to make controls on unclassified results more explicit. Their chief recommendation was that academic science — unlike hardware or goods of commerce — should not be regulated by means of export controls applied after the fact, but rather by means of restrictive clauses in the initial research contracts. The universities agreed that contracts could require results to be submitted to DoD for review and comment at the same time as they are submitted for journal publication and, apparently a new concession, that restrictions could be placed on the participation of foreign nationals in sensitive research projects. In the forum's view, these restrictions would be invoked only for a small "grey area" of sensitive unclassified research and only on the recommendation of the DoD programme officer directly involved — presumably the official most knowledgeable about the science.

Wilson said that their recommendation also made clear that national security needs would have to be weighed against the wish to maintain scientific openness. The universities, many of which forbid acceptance of classified research or any research contracts that restrict freedom to publish, have been adamant on this point.

But the forum's recommendations may not carry weight. Wilson said that under a

directive issued late last year (known as 2040XX), authority to regulate technology transfer — including publication or dissemination of scientific results — was given to DoD's Undersecretary for Policy (seen as a hard-liner) rather than to the Undersecretary for Research and Engineering, who is responsible for basic research contracts. Wilson says the participation of policy officials on the forum has been reluctant at best.

Complicating the picture within DoD is the work of a senior interagency group on technology transfer, chaired by an official

of the National Security Council. This group is attempting to overhaul the fragmented and not infrequently contradictory government-wide policy on export controls, currently spread across 14 cabinet-level agencies in 44 offices. This group is expected to report early next year, and its policy recommendation would supersede any internal DoD policy. But Wilson and others say their chances of coming up with a coherent policy are not great.

Meanwhile, no action has yet been taken by DoD on the forum's recommendation. Yet another DoD committee, under the chairmanship of Edith Martin of the Research and Engineering Office, is now attempting to formulate an official DoD policy on the issue.

Stephen Budiansky

Scientific fraud

The system defends itself

Detroit

THE co-author of a controversial new book claiming that fraud and cheating are widespread characteristics of academic science was given a predictably hostile reception over the weekend when he carried his message to the annual meeting of the American Association for the Advancement of Science (AAAS) here.

Mr Nicholas Wade, editorial writer for the *New York Times* and a former deputy editor of *Nature*, told a special session of the meeting that the spate of recent episodes in which respectable scientists had been discovered doctoring their research or plagiarizing that of others could not be explained by individual pathology alone. "It is not just a matter of rotten apples in the barrel", he said, "but something to do with the barrel itself."

Pointing out that new cases of fraud were now coming to light at the rate of about one a month, Wade said the discovered cases could represent only the tip of a very large iceberg. And he noted that in none of the cases had the fraud been uncovered by the traditional checking mechanisms of science — peer review, the refereeing of papers before publication and the replication of experiments.

Yet precisely these mechanisms, he argued, are used by scientists and philosophers of science to argue that research fraud is rare and, once committed, certain to be discovered. In practice, however, cheating is virtually never detected by peer reviewers or referees, and few scientists bother to replicate experiments from the published literature. The opportunities to cheat, Wade added, are becoming more frequent because of the readiness of laboratory chiefs to put their names to papers prepared by junior colleagues with little or no supervision.

Wade's argument was angrily challenged by Norton Zinder, professor of microbial genetics at Rockefeller University, New

York, who said science would not have survived or prospered if cheating were as prevalent as Wade's book, *Betrayers of the Truth*, alleged.

Zinder went on to accuse journalism of being the only profession in which fraud was prevalent, and quoted a succession of sensational medical headlines from the *National Enquirer* and companion publications to prove his point.

Zinder claimed that the attempt in *Betrayers of the Truth* to argue that science could be studied by studying its pathology was "a pretentious fallacy". Fraud was common in all walks of life and the fact that some scientists were fraudulent explained nothing about the structure of science itself. At least six of the recent cases of fraud were committed by individuals who turned out to be psychopaths who could have cheated in whatever profession they had entered.

Zinder also rejected Wade's criticisms of the traditional checking mechanisms in science. These were never intended to detect fraud but they were inexorable, he said. The fraudulent scientist had to live with the perpetual fear that he would eventually be discovered. To devise an efficient checking mechanism would, in any case, destroy everything that was good about science because it would destroy trust between colleagues.

Betrayers of the Truth received only slightly gentler treatment from other panellists. Daryl Chubin, a sociologist from the Georgia Institute of Technology, said the "cocksuredness and self-righteousness" of the book rankled, although it was an effective assault on the conventional ideology of science. Professor Richard Westfall Jr, a historian of science from the University of Indiana, said the book attacked a straw man — nobody still believed science to be a completely objective procedure in which the checking mechanisms worked perfectly.

Peter David

UK universities

One way to keep spirits up

Salford (Lancashire)

How does a university best adjust to a reduction of its budget by a third? The answer that the University of Salford seems to have stumbled on is to behave as if innovation is still the order of the day. In the process, the university seems well on the way to becoming the most market-oriented of British universities, second in this respect only to the Cranfield Institute of Technology.

It has also earned a reputation for outspokenness of a kind expected of a North of England city which other universities fearfully admire. For Salford's vice-chancellor, Dr John Ashworth, has taken to saying publicly what some of his colleagues elsewhere only whisper — that the University Grants Committee (UGC) should be abolished.

Biting the hand that chose not to feed one inevitably smacks of sour grapes, but the consequences for morale have been remarkable. Ashworth, then a member of the British central government's think tank, had been appointed to Salford before UGC announced its plan to cut Salford's budget by a third in July 1981, and took office only the following September. Always talkative, he seems to have been talking ever since.

The strategy seems to be that of combative acquiescence. Ashworth says he told his academic colleagues, in his first few weeks, that they should not look to UGC to soften its hard line. "That's what the traditional universities think of us, and we've got to make our way without their approval." Ashworth and his academic colleagues are still puzzled to know why UGC dealt so severely with what was even then fashionably a predominantly technological university, although students' relatively poor school-leaving grades may have played a part in the decision.

The consequence is an undertow of bitterness beneath the elation of the past eighteen months. From Salford, UGC is regarded as a poorly staffed and badly administered London outfit, whose decisions are made by miscellaneous academics from traditional universities tainted themselves with prejudice. "We couldn't last if we were so badly run."

Ashworth's innovations will not make good the shortfall of the UGC subvention, but are enough to persuade academics that innovation can occur in the face of adversity. Selling academic services is one theme, to which end Salford has surprised many comparable and traditional British universities by offering training for overseas diplomatic staffs, primarily from a base in London. There is also a contract (financed by the European Community) by means of which the university, jointly with the North Wales College of Higher Education at Chester, is helping to train staff and techni-

cians at the new Jordanian university at Yamuk.

Domestically, Salford has with some success revived a long-standing industrial centre, providing consultancy services to companies in the North-West of England. Most of the business is in microprocessors and manufacturing technology, and the centre now employs a staff of more than thirty and is looking forward to a turnover of £2 million.

"Campus" is the code name for another innovation by means of which industrial companies subscribe for membership in a kind of contact club, an arrangement by which companies can be introduced to academics who might help with specific problems.

Perhaps the most imaginative new venture, however, is the "matriculation unit" — a small academic unit preparing overseas students for conventional undergraduate courses, mainly by the provision of English instruction but also with help in mastering the local supermarkets.

Collectively, these developments appear to demonstrate that almost any change can be enlivening. There will be more high jinks later in the year, when contractors will begin installing in the River Irwell (a muddy tributary of the Mersey flowing through the campus) prototypes of two power generators working on a metre or so of water head. The cost of £200,000 will be met through some kind of external corporate structure.

Illustrations such as these show potentially displaced academics that even if UGC will not pay their salaries, there is always a welcome for them in the role of entrepreneurs. Even the students' union is said to have caught the mood, offering to let its university subvention be cut by 40 per cent in return for the right to set up student enterprises. There is now a sports shop alongside the squash courts and talk of buying up a pub.

Whether the revival of morale engen-

dered by all this change will survive the crunch that lies ahead remains to be seen. The academic staff must shrink to 340 (from 480) by September 1984, and some of those displaced are unlikely to become entrepreneurs overnight. Salford academics are especially angry that UGC, which offered a year's extension of the target date for reducing home student numbers to 3,000, has not abated the severity of its budget cuts so that teachers can be paid.

Meanwhile, Ashworth has figured out, to have UGC as an enemy is not an unmixed curse. Ideally, there should be a multiplicity of sources of financial support. If UGC turns sour, surely the chance that others will appear on the scene can only be enhanced? The calculation is well-judged. Ashworth has successfully taken the lead in persuading the British Department of Industry to talk to a group of universities like his own. (That conversation has been postponed pending the outcome of the election.)

A sense of being isolated in the North of England flourishes, as does the accompanying sense of being independent. Salford was last month awarded two of the British Government's "new blood" posts, opportunities (and funds) to appoint young academics to predominantly research posts. Ashworth is offended that on the heels of a letter from UGC announcing the good news, he received another from a research council asking him to appoint a nominated external moderator of the appointment "who has agreed to serve". "Quote me as saying I'm very angry", Ashworth says. Probably, however, he will do as asked.

Ashworth's colleagues accordingly ask repeatedly when he will stop berating UGC. They suppose that with the replacement of the present chairman, Dr Edward Parkes, at the end of this month by Sir Peter Swinnerton-Dyer, there will be some kind of honeymoon. The conventional wisdom is that it will last for six months. The more accurate calculation is that the truce will not endure beyond the point at which Salford again needs a sense of having to battle for its survival. **John Maddox**



Salford, hard hit and mystified by UGC cuts

AIDS not a "social disease"

SIR — The author of "No need for panic about AIDS" (*Nature* 28 April, p.749) wrote against the temptation to see AIDS as a twentieth century Sodom and Gomorrah. Strange then, that by the end of the article she/he could not resist the temptation of standing in the place of God and hurl fire and brimstone at gay men. Shortly after commenting that AIDS was not "especially infectious" the author goes on to imply that gay men are a "threat to public health" because of their "pathetic promiscuity". After offering no evidence that the recreational use of drugs such as amyl nitrite is implicated in AIDS, the author suggests that their use be discouraged. Finally, the writer suggests that gay men change their lifestyles, concluding with the thought that "the fear of AIDS will no doubt help" in turning us all from sinful paths.

Such tedious moralism might well suit the pages of a polemical religious tract but it ill-becomes *Nature*.

JONATHAN GREVESON

London E8, UK

SIR — Your News and Views article, "No need for panic about AIDS" (*Nature* 28 April, p.749) is at best unhelpful. To begin at the end, in the last paragraph innuendos in phrases such as "pathetic promiscuity of male homosexuals" and assisting "the mechanics of male homosexuality" suggest that there might be a bias in the piece along the lines that panic about AIDS could be expected to increase in proportion to the effect of the epidemic on heterosexuals.

The writer also seems to be unaware of reports from Belgium (*Lancet* 19 March, p.642) and France (*Lancet* 26 March, p.700) of AIDS in black Africans, particularly natives of, or visitors to, Zaire. None of these people, incidentally, were known to be homosexuals. These data and the incidence of AIDS amongst Haitians ought surely to be looked at in the context of another *Lancet* report (12 March, p.545) that solarium exposure can produce immunological disorders in otherwise normal (and presumably heterosexual) subjects analogous to those in AIDS patients.

Following a suggestion by Dr Alex Comfort (*Br. J. sexual Med.* September 1982, p.4), moreover, research in Los Angeles amongst male homosexual volunteers has demonstrated a connection between homosexual behaviour, specifically the passive role in anal intercourse, and immunodeficiency, though the T-cell imbalance differs from that characteristic of AIDS (*Lancet* 19 March, p.609). Both the authors of this report and the work of three New York physicians, Sonnabend, Witkin and Purtilo (unpublished in March 1983), found evidence that allogeneic semen may promote immune suppression.

Nor does the author note the significant difference between the number of AIDS cases reported in the United States and in

Europe. With 29 in France (a minority being male homosexuals), 5 in Belgium, 14 reported so far in the United Kingdom, and between 10 and 20 in the rest of Europe, the imbalance is striking. Any attempt to understand AIDS short of the isolation of a new infectious agent must explain why two regions with similar cultures, populations and numbers of homosexual men should be so differently affected. I have learned to expect more from *Nature* than this partial and prudish review.

RICHARD B. FISHER

London N1, UK

SIR — As a statement about what we know and don't know about AIDS, your piece "No need for panic about AIDS" (*Nature* 28 April, p.749) is muddled and confusing. It also is dated and not well-informed.

But I am alarmed that a journal with the high standing that *Nature* enjoys in the scientific community could possibly say AIDS is a "social problem". Or that AIDS can be described simply as "disturbing" (rather than, say, frightening) in light of the high mortality involved.

And how on earth can you say that we needn't be deeply concerned about AIDS because "the numbers of people among whom the disease has been recognized in the past few years is of the order of a thousand most of whom are male homosexuals". How many human beings must be afflicted — how many people must die — before we need to pay attention to a disease . . . or a "social problem"? Or are numbers of human beings not important because most of those affected can be labelled "male homosexuals"?

And tell me, pray, why the vicious opinions expressed in the final paragraph of the piece belong in any scientific journal, much less in *Nature*? The hatred of "male homosexuals" distilled in phrases such as "male homosexuals should be persuaded to change their ways", "the pathetic promiscuity of male homosexuals is the most obvious threat to public health" and "use of substances such as amyl nitrite to assist the mechanics of male homosexuality" is a disgrace to the trust that your readers place in your publication.

JOHN TERRELL

State University of New York
at Binghamton,
New York, USA

● Not unnaturally, this is a contentious subject. The starting point for the article complained of was the danger, still extant, that proper concern about the origin of AIDS may engender public alarm. Nobody suggests that the causation of AIDS is anything but a medical and scientific problem, but is the "social problem that has arisen" — for blood transfusion services — negligible? Similarly, nobody expects that male homosexuals will cease to be homosexual, just that they should be aware of the dangers of promiscuity and of the use of amyl nitrite. — Editor, *Nature*.

Sterling and research

SIR — Your leading article, and the report by Philip Campbell (*Nature* 5 May, pp. 1 and 4) rightly draw attention to a deplorable state of affairs. Research programmes currently relying on Science and Engineering Council (SERC) support are to have funds suddenly withdrawn (about £1 million from each board of SERC), and the whole future of particle physics research is placed in grave jeopardy. All this is a result of the fall in the value of sterling which has raised SERC's international commitments by about £4 million this year. Worse may follow, since changes in contributions which are related to gross national product could lead to an even greater deficit.

Of the international contributions paid from the SERC budget, the largest goes to CERN, the laboratory at Geneva which furnishes the very large and expensive machines necessary for experiments in particle physics. SERC takes the view that short-falls in international subscriptions should be "recovered" from the budgets of the appropriate research areas. If present estimates are correct, the effect on particle physics research in the coming years would be devastating: the support British university physicists depend on for their experiments would be about halved, forcing them to break commitments made with physicists of other countries to share in the cost and effort of doing experiments, and seriously damaging preparation now under way across Europe to use the new accelerator, LEP, being built at CERN.

CERN is not a remote money-consuming international organization, it is *our* laboratory for experimental particle physics. Like most of our nearly 2,000 colleagues from European universities who use CERN, we have no facilities for this research in our own country. Britain has helped, through our contributions over 25 years and the efforts of our physicists, to make CERN what it is today — the leading laboratory in its field in the world. This is an achievement the country, and SERC, should be proud to share; it is a great success which deserves enthusiastic support, not grudging acceptance because it is too difficult to leave!

SERC should shoulder its responsibility for basic research in the universities and secure a guarantee that the Treasury will provide the currency committed to international subscriptions, as is the case in most other countries.

With respect for the authority of your journal, I cannot believe that Mrs Thatcher — who has shown a warm interest in the recent discoveries at CERN — really accepts that basic research in the universities should be such a desperate hostage to the capricious fortunes of sterling.

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The legend of Darwin's finches

SIR — Mark Williamson¹ has taken issue with a statement made by Peter Boag² in his report on the Linnean Society symposium on "Evolution in the Galapagos". The statement, drawn directly from my contribution to the symposium³, is that "Darwin . . . omitted any mention of the finches [of the Galapagos Islands] from his *Origin of Species*". Williamson points out in rebuttal that Darwin alluded indirectly to the finches in the *Origin* (1859) when he stressed the highly endemic nature of the Galapagos birds and fauna as a whole.

Williamson, who did not attend the Linnean Society symposium and who had not read my previously published papers on this subject (cited in Boag's report⁴), fails to mention that many of the quotations presented in proof of his contention were also cited by me — both in my symposium contribution and in my publications. Thus readers of Williamson's letter to *Nature* are unfortunately misled into thinking that I (and Boag) were unaware that Darwin was implicitly including the finches when discussing in general terms the birds of the Galapagos Islands.

Of more significant historical importance, however, are several other misconceptions inadvertently created or perpetuated by Williamson. He claims, for example, that Darwin had already provided "sufficient detail" about the Galapagos finches in his *Journal of Researches* (1839, 1845) and that it was therefore quite unnecessary for him to cite the famous finches again in the *Origin*. In other words the omission of the finches from the *Origin* is taken as historical evidence in support of how famous these birds had become by 1859. In actual fact, not a single biologist between 1839 and 1859 (except Darwin) is known to have discussed the unusual case of Darwin's finches; and Darwin could hardly assume that readers of the *Origin* would, as Williamson suggests, be "well aware [like Darwin] of the importance of this famous group of birds".

Thus the point of real historical interest is why Darwin, who surely wanted to bolster the text of the *Origin* with his most convincing scientific evidence, chose to omit any specific reference to a group of birds that he supposedly thought were so important for his evolutionary argument. The answer to this question, discussed by me elsewhere in more detail⁵, is threefold. First, Darwin's island localities for his Galapagos finches were almost entirely borrowed after the *Beagle* voyage from the fully labelled collections of three other *Beagle* shipmates. Based upon this borrowed evidence, Darwin had suggested in his *Journal* (1845) that four (and perhaps more) of the finch species were confined to different islands. But he was rightfully distrustful of this published claim, which, being based on his inaccurately copied locality notes and insufficient collecting,

was totally in error. Darwin could not therefore cite the finches in the *Origin* as a convincing case of evolution by geographic isolation; and he well knew it.

Second, with the exception of 2 species of Galapagos finches, Darwin mistakenly thought the remaining 11 species named by John Gould⁶ had identical diets. For this reason he could not argue that the finches, with their various sized beaks, were the product of evolution by natural selection, character displacement, and ensuing adaptive radiation. Other ornithologists remained in doubt on this question until David Lack's famous book *Darwin's Finches* turned the tide in 1947⁷.

Finally, Darwin was personally unconvinced that all of the Galapagos finches, particularly the warbler finch (*Certhidea olivacea*), were descended from just one ancestral colonist, although he had previously alluded to this possibility in his *Journal* (1845), in a veiled evolutionary reference. Darwin's caution was well advised. During the remainder of the nineteenth century, ornithologists generally considered Darwin's finches to have descended from two or three different ancestors — a warbler, a ground finch, and a separate form that gave rise to the 6 species of *Camarhynchus*. This issue of ancestry was not resolved for 50 years after the *Origin of Species* was published.

It is for these three reasons that Darwin made as his paradigmatic example of evolution in the Galapagos the mockingbirds (*Nesomimus* — which Gould had distinguished as three species inhabiting four separate islands⁸) rather than the complex and more debatable case of the finches. Significantly, the Galapagos finches were briefly mentioned in Darwin's "Big Book" on evolution, written between 1856 and 1858. But Darwin chose to delete his speculative reference to the finches' possible common ancestry when Wallace's famous letter (1858) prompted him to abstract his manuscript down into the *Origin*. He evidently felt that the more general he made the Galapagos discussion (especially regarding the finches), the better off he was. Yet the legend later arose, and continues to live on, that Darwin's finches not only converted Darwin to the theory of evolution — eureka-like — but also "deservedly held a prideful place in the *Origin*"⁹.

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1. Williamson, M. *Nature* 302, 566 (1983).
2. Boag, P.T. *Nature* 301, 12 (1983).
3. Sulloway, F.J. in *Evolution in the Galapagos* (ed. Berry, R.J.), Biol. J. Linn. Soc. 21, (in the press).
4. Sulloway, F.J. *J. hist. Biol.* 15, 1 (1982); *Bull. Br. Mus. nat. Hist. (Zool.)* 43, 49 (1982).
5. Sulloway, F.J. *J. hist. Biol.* 15, 325 (1982).
6. Gould, J. *Proc. Zool. Soc. Lond.* 5, 4 (1837).
7. Lack, D. *Darwin's Finches* (Cambridge University Press, 1947).
8. Gould, J. *Proc. Zool. Soc. Lond.* 5, 27 (1837).
9. Ruse, M. *Darwinism Defended*, 115 (Addison-Wesley, Reading, Massachusetts, 1982).

Solar constant

SIR — In their review "Changes in the solar constant and climatic effects", Eddy *et al.*¹ raise the issue of whether the sunspot-associated irradiance dips now seen in satellite radiometry were detectable in the Smithsonian (APO) ground-based radiometry carried out between 1923 and 1952. Two recent analyses of the APO data (still the longest set of solar constant data) reach opposite conclusions.

The point of view put forward by Eddy *et al.* was that the solar irradiance changes caused by magnetic activity were too small to be detectable in the APO data. This conclusion was based on the extensive analysis by Hoyt², which found no significant evidence for an irradiance modulation by solar activity in the APO solar constant values. A different conclusion was reached in a separate study of the APO data base by Foukal *et al.*^{3,4} providing evidence for a short-term modulation of irradiance by spots and faculae at a combined r.m.s. level of $5-7 \times 10^{-4}$.

A number of considerations should be weighed in evaluating this disagreement. On one hand, Hoyt's analysis has shown that a substantial fraction (20–50 per cent) of the total variance seen in the APO data can be attributed to inadequate correction for atmospheric aerosols and dust. His analysis also showed that caution must be exercised in deriving confidence limits from that database, since straightforward tests for the influence of spots on irradiance measured at different APO field stations can yield contradictory results at similar levels of formal significance.

On the other hand, the modulation reported by Foukal *et al.* is at a level below 10 per cent of the total variance of the APO irradiances, so its presence is not excluded by the finding that 20–50 per cent of the total variance is atmospheric noise. We note also that the irradiance increase with facular area, irradiance decrease with spot area, and the combined level of 28 day irradiance modulation by magnetic activity, are three independent results of the Foukal *et al.* study. Their good agreement in sign and amplitude with recent results of radiometry from the Solar Maximum Mission and Nimbus-7 deserves attention.

It is still unclear whether or not C.G. Abbot's epic measurement programme detected real solar irradiance variations. The evidence for both points of view is presented in the publications cited below.

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1. Eddy, J.A., Gilliland, R.L. & Hoyt, V.H. *Nature* 300, 689–693 (1982).
2. Hoyt, D. *Rev. Geophys. Space Phys.* 17, 427–458 (1979).
3. Foukal, P., Vernazza, J. *Astrophys. J.* 215 952–959 (1977).
4. Foukal, P. & Vernazza, J. *Astrophys. J.* 234, 707–715 (1979).

New definition of the metre

B.W. Petley*

Some of the physical assumptions which are implicit in the proposed new definition of the metre are discussed, including the mass of the photon and the constancy of the fine structure constant.

It is probable that the next meeting of the *Conférence Générale des Poids et Mesures* (CGPM), to be held in October 1983, will agree to a change in the definition of the metre. I now discuss the wording of the proposed definition, which effectively fixes the magnitude of the velocity of light, and some of the underlying physics, including the photon mass and the constancy of α .

As a background to the reasons for the change, it is seen from Fig. 1 that the accuracy with which we are able to measure the velocity of light has increased dramatically over the centuries. It is evident from Fig. 1 that the post-war measurements approached and then exceeded the accuracy with which the metre could be realized using the metre bar. Similarly the present generation of measurements is limited by the accuracy with which the metre can be realized using the specified orange radiation from a krypton-86 lamp. This limitation is also true of other quantities involving precise length measurement such as the Rydberg constant. Thus the advent of highly stable lasers, whose reproducibilities exceed that of krypton-86 lamps, and the demands of astrophysical measurements such as lunar and planetary ranging (with fractional accuracies $\sim 10^{-12}$) which will continue into the next century, have increased the pressure for an improved definition of our unit of length.

One possibility would be to change to a definition in terms of a specified laser-induced transition but, because the ultimate 'best method' has not necessarily been found, this option would almost certainly lead to the position where it was necessary to make further revisions each time that a 'better' transition was discovered. After careful consideration of the possible alternatives, the *Comité Consultatif pour la Définition du Mètre* (CCDM), have proposed an equivalent, but more satisfactory solution, whereby the specified laser radiations are involved in the practical method of realization of the definition rather than in the definition

itself. They recommended that the metre be redefined as follows (Recommendation M1, 1982):

"Le mètre est la longueur du trajet parcouru dans le vide par la lumière pendant une durée de 1/299,792,458 de seconde."

This is the wording of the French text that will be eventually adopted by CCDM, CCU (*Comité Consultatif des Unités*) and CIPM (*Comité International des Poids et Mesures*) after it has been discussed, and most probably adopted, by the CGPM in October 1983. The above wording is the result of discussions of various alternatives that have been considered over the past decade by the CCDM (see refs 1 and 2). A second recommendation accompanies the above, abrogating the former definition:

"la définition du mètre en vigueur depuis 1960, fondée sur la transition entre les niveaux $2p_{10}$ et $5d_5$ de l'atome de krypton 86, soit abrogée."

French is the definitive language of the International System of measurements (SI) and so any English translation must be considered with care, but the following is expected to be the internationally agreed English version:

"the metre is the length of the path travelled by light in vacuum during a time interval of 1/299,792,458 of a second."

The wording of this short and succinct definition, which is discussed here, is a compromise for it was necessary to find a form of words suitable for all purposes; one which could be understood by those in schools, would suffice for the requirements of legal metrology and also satisfy the sophisticated demands of modern metrology and science, including relativity and cosmology, at the highest levels of accuracy.

The word 'light'

One surprise in the wording may well be the use, which we now discuss, of the word 'light' rather than 'electromagnetic radiation'. One reason lies in the requirements of legal metrology in some countries where the definition must also convey an idea of the method of realizing the unit. A further important reason lies in the need to ensure that the demands of modern science do not

lead to theoreticians using a slightly different physical quantity, for what is termed 'length', than the experimenter.

In the proposed definition it might be thought that 'light' is the radiation which can be 'seen' with the eye and, taking the CIE curves³ (*Commission Internationale de l'Éclairage*), this would approximately cover the range 400–700 nm. Such a range is perfectly acceptable in the definition, but would be too restricted for metrological purposes concerned with the realization of the definition since the methane-stabilized helium–neon laser, for example, lies in the infrared region of the spectrum. It may be that in the future we might choose instead to interpret 'light' as the radiation emitted by a 'laser', for it is a word that originated as an acronym for light amplification by stimulated emission of radiation. The word 'laser' is used at present to describe devices which operate between the far infrared (the HCN laser at 0.8 THz) through to the X-ray region or even to the γ -ray region (see, for example, ref. 4). The very important microwave region, which is used for radar measurements of distances and also for the measurements of interplanetary distances (with resolutions in the region of 3 cm), is only just outside the range of frequencies covered by the above definition of the word 'light'. Often the word 'light' is taken to mean any electromagnetic radiation, as when discussing relativity or the velocity of light. This is a much more general interpretation, which would encompass all frequency regions.

What objection can there be to the use of the phrase 'electromagnetic radiation'? In truth, of course, there need be none if we so choose, but in the science of today the phrase 'electromagnetic radiation' includes radiation having frequencies that extend from microhertz to the very high-energy γ -ray region of the spectrum. As far as low frequencies are concerned, difficulties arise because measurements can only set upper limits on the mass of the photon and corresponding limits to any possible dispersion in the velocity of light; see ref. 5. The present limit on the photon mass is thought to be essentially equivalent to a photon frequency of < 0.25 Hz (see refs 6 and 7; we can, of course, generate lower frequencies in the laboratory). This limit is set via different null experiments in electromagnetism and, as with all null experiments, the assumptions must be carefully examined. However, the phrase 'electromagnetic radiation' would have to

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be qualified by specifying a lower frequency limit: such a limit might have to be revised as it was examined more sensitively. At the other end of the electromagnetic spectrum the question of the possible granularity of space and time arises — for they may be quantized. These effects are expected to occur in the region of the Planck–Wheeler length $(G\hbar/c^3)^{1/2}$, $\sim 10^{-35}$ m, and the Planck time $(G\hbar/c^5)^{1/2}$, $\sim 10^{-43}$ s. Thus we might well have to set upper frequency bounds for the electromagnetic radiation as well.

It is apparent, therefore, that, if we wish our length unit to be defined unambiguously at the part in 10^{14} level of accuracy, there are considerable advantages from the use of the word 'light', instead of 'electromagnetic radiation' in the definition: to the layman as well as the scientist. It is quite in order to use electromagnetic radiation of any convenient frequency for the realization of the definition — as long as it can be shown that it either has the same velocity as light or that an appropriate correction can be made.

The word 'vacuum'

Another interesting word is 'vide' or 'vacuum'. We are moving into an era where interpreting 'vacuum' as the mere absence of atomic matter in the direct path of the light might not suffice for the realization of our unit of length with the highest accuracy. Thus, in the foreseeable future, with the aid of laser sources (or even controlled nuclear explosions), we may well be able to produce sufficiently intense sources of radiation to change the velocity of light in the local vacuum. Equally, on a more cosmological scale, there are distortions of space in the vicinity of massive bodies such as stars and the 'vacuum' might well contain an otherwise invisible black hole. With this type of effect in mind it has sometimes been the preferred practice in English to use the phrase 'free space' rather than 'vacuum' — although the phrase has no well defined meaning for the definition. However, the definitive language is French, in which it appears that 'vacuum' and 'free space' have no separate usage.

Note that, in the cosmological context, the definition does not specify the straight line distance, but simply the distance travelled by light. This allows for possible curvature of the space metric and suggests that care may be required in some relativistic problems — but no more so than with the earlier definitions of the metre.

A surprising notion is that we might not wish the vacuum to be totally empty after all. The reason for this is that, as has been pointed out by Primak and Sher⁸, the present upper limit on the mass of the photon of $<10^{-16}$ eV is established⁹⁻¹⁶ for photons which are bathed in radiation whose 'temperature' is at least that corresponding to the black body background radiation in the Universe of ~ 2.1 K. They have hypothesized that a phase transition might be

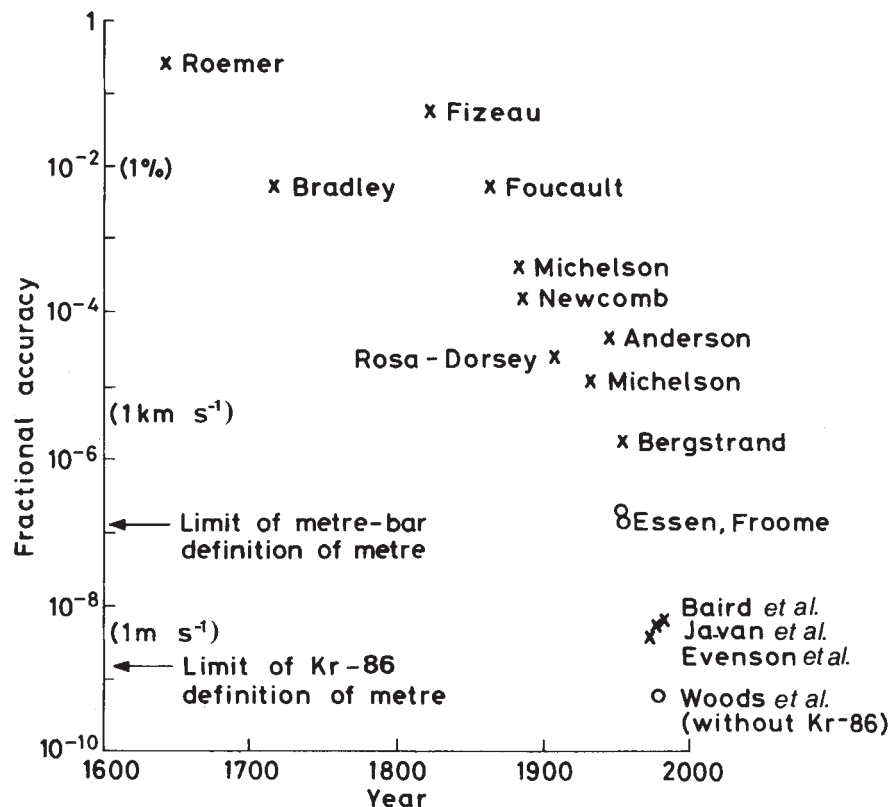


Fig. 1 Accuracy with time of determinations of the velocity of light.

possible at lower temperatures, thereby resulting in a photon mass which could be as large as 10^{-4} eV, corresponding to a frequency of about 24 GHz. Such a photon mass would be a major embarrassment, but not catastrophic, for the definition and the word 'light', as discussed above, would have to be defined more closely. However, their hypothesis is still the subject of debate^{14,15}, but it is evident that it may remain convenient to leave the question of what is meant by the word 'vacuum' in the definition rather undefined at present — it might be the vacuum of otherwise empty intergalactic space.

The phrase 'time interval'

At first sight the use of the concept of a time interval in the definition causes relativists some alarm for it might lead to problems of realization through the need to transfer a clock from one end of the metre path to the other (see, for example, ref. 16 for a discussion of relativistic experiments with clocks). This is a problem of realization, not of definition, and so can be overcome in the method of realization. Thus for the implementation of the ampere we do not need to use the infinitely long straight and parallel wires of the definition and we are allowed to curl them up into a helical solenoid in a manner consistent with the definition. Similarly there is no need to move clocks for the metre definition and the light may traverse an 'out and back path' in an interferometer.

The latter method leaves some cause for

disquiet to those who worry that our new unit might not be commutative, that is the time for light to travel from point A to point B might not equal the time to travel from point B to point A (if the out and return paths effectively enclosed an area it might well, of course, be necessary to make a correction for the Sagnac effect). It is true that most of the measurements of the velocity of light since those of Roemer have been out and back, and not unidirectional, so that one should not ignore the possibilities of some asymmetry entirely (see refs 17 and 18). However, it is rather unlikely that, even if there were a real effect, we would choose to use other than the mean of the two directions for our metre definition. In this connection it may be worth pointing out that our present realization of the second is made in the rotating reference frame of the Earth and that a relativistic correction is required for some experiments which are performed in space. (Bertotti¹⁹ discusses relativistic effects in connection with time scales.) Similarly, our realization of the metre may apply in the rotating frame, and care may be required in connection with space experiments — it will depend on which 'second' is used and one will probably need the 'local' second to realize the 'local' metre.

The word 'travelled'

A further aspect of the definition lies in the use of the word 'travelled', for in using this word it appears that the concept of length is derived after the prior concept of travel-

ling. The question may well arise as to whether, in view of this, we can continue to regard velocity as being a derived unit. Thus it could be that we might choose to regard velocity as being defined simultaneously with length (in the same way as μ_0 is essentially defined with the ampere), or even that velocity rather than length was the base unit. This question will be for the global users of SI units to decide when they have had experience with using the new definition. However, it is quite in order to have a qualitative appreciation of the physical quantities required for a description of the Universe before their magnitudes are defined: the ordering of our concepts may differ from the ordering of the definitions of the units of measurement. The concept of light travelling also makes it clear that it is the group velocity and not the phase velocity of light that is implicit in the definition, moreover it avoids the necessity to specify the curvature of the wavefront (plane, spherical waves and so on) for such questions are relegated to the realization of the definition.

The number

Considered in the abstract, it seems a pity to saddle posterity with the complicated number 1/299,792,458 and, viewed from the twenty-first century, our descendants may well wish that a simpler number had been chosen. Unfortunately there are probably already too many people using the above number for any change to be made. Thus the CCDM in Recommendation M1, 1982 considered that "there is an advantage, notably for astronomy and geodesy, in maintaining unchanged the value of the speed of light recommended in 1975 by the 15th CGPM in its Resolution 2 ($c = 299,792,458 \text{ ms}^{-1}$)".

Whenever a definition is changed or modified it is, of course, essential to ensure that the magnitude of the unit remains unchanged within the previous reproducibility of the unit. The relative uncertainty of the krypton-86 definition of the metre is about $\pm 4 \times 10^{-9}$. With hindsight one can argue that it might have been preferable, without stretching the statistics too much, to have rounded up the last digit (that is the 58 to 60) — thereby allowing a digit to be dropped (there is a possibility that some users will do so anyway since many of the present generation of hand-calculators and microcomputers have only eight-digit accuracy — indeed, this may provide a more subtle source of error in the future than did the use of 300 to convert from electrostatic volts to volts in the days of the cgs system). There is perhaps an advantage in specifying c exactly in metres per second, in that one is less likely to lose the zero — thereby being out by a factor of 10! Overall, the widespread access to sophisticated computing power enjoyed by an increasing fraction of the population makes the argument for a simplified number more one of aesthetics

than practical metrology — except perhaps that it encourages the image of the metrologist as one who makes simple measurements unnecessarily complicated.

Duration of the definition

The definition of the metre has changed several times and it is interesting to recall where our present-day metrology would be if we had retained the original definition in terms of a ten-millionth of a specified quadrant of the circumference of the Earth, Fig. 2. All of those who have taken part in the formulation of the elegant new definition hope that it will endure, and there is no reason to suppose that they are wrong to do so. However, there are two interesting precedents. The first is the comparatively short duration of the krypton-86 definition of the metre. The gestation period was much longer for it took more than a century to progress from the initial suggestion of Babinet in 1829, and the associated pioneering nineteenth century experimental work of Benoit, Michelson, and others, through to the adoption of

1960. It must be recognized that, although the definition has only endured for 23 years, it has played a major part in the development of length metrology: initially by providing a more stable reference against which length measurements could be compared and more recently by giving a measurement target that was within the capability of metrologists to surpass. In 1960 it was not possible to sit and wait for a better method to come along. More and more scientists outside the various national metrological laboratories were finding the metre bar definition an obstacle to communicating the results of their measurements to one another with the requisite accuracy. The reasons for change in 1960 were therefore severely practical rather than primarily aesthetic and similar considerations obtain today. The second precedent comes from theoretical physics. In this area the natural units are the fundamental physical constants and it is often more convenient to use non-SI unit systems during the calculations even if the conversion to SI units is made at the end. Thus unit-system defining statements of the

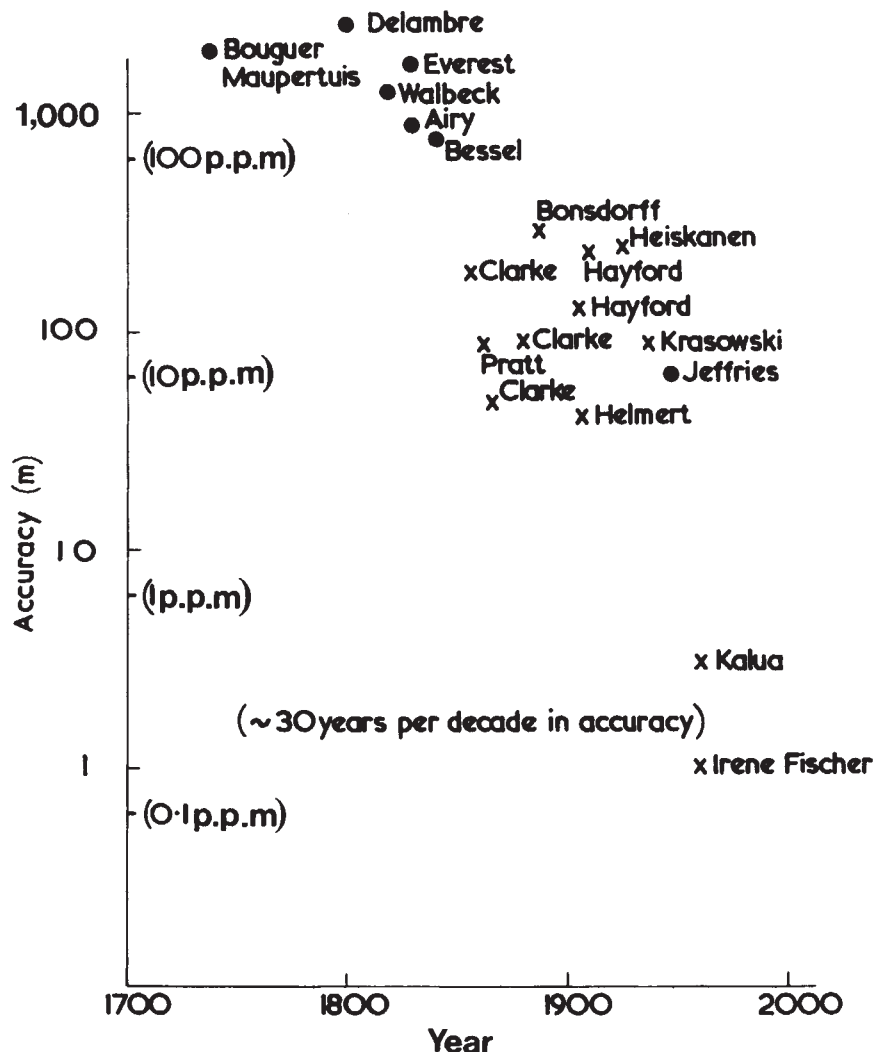


Fig. 2 Estimates of the equatorial radius of the Earth from arc measurements; fractional deviation from IAU-62 value of 6,378,160 m (x, above; ●, below IAU value).

form:

$$\text{let } e = \hbar = c = 1$$

are frequently encountered in theoretical papers²⁰. It is interesting that there is no unique length unit in such systems (or time unit either — as Dirac²¹ discussed in setting up his 'big number' cosmological model): each system has its own 'natural' length unit (equally the above system must be used with care in discussing certain relativistic problems since it, along with the new metre definition, makes c an invariant quantity). However, the natural length units²¹ are related by the dimensionless fine-structure constant and one may construct what is known as the 'Bohr ruler' (thus 'natural' atomic length units might be the classical radius of the electron, the Bohr radius of the atom, or the reciprocal of the Rydberg constant R_∞ , and so on).

If there is no unique length unit in such systems then might not the same be true for our practical metrology²²? If we look at the evidence we see that the metre bar, the krypton-86 definition of the metre and now the change to a definition which essentially involves a hyperfine atomic transition (through the use of the caesium-133 definition of the second), are almost certainly all related by the product of the reciprocal of the Rydberg constant and the dimensionless fine-structure constant raised to different powers — at least to first order. Thus the length of the metre bar depends on the size of atoms — which in turn depend on the Bohr radius $a/4\pi R_\infty$, the krypton-86 wavelength is characterized by R_∞^{-1} and the wavelength corresponding to the specified caesium-133 transition is characterized by $\sim \alpha^2 R_\infty^{-1}$ (these relationships are more readily seen if one considers, for example, the wavelength of the atomic hydrogen Balmer- α line in the visible and the wavelength associated with the microwave transition used in the hydrogen maser $\sim \alpha^2 R_\infty \mu_p/\mu_B$, as being equivalent to the wavelength of the specified orange radiation from krypton-86 and the radiation from the specified hyperfine transition in caesium-133 atoms respectively — the factor with the latter becomes $\alpha^3 R_\infty^{-1}$ if we characterize μ_p/μ_B by α as well).

This raises an interesting point, for the constancy of the fine-structure constant is something which is still discussed²³⁻²⁵, although not at a level likely to be of significance in our length metrology (for example, see ref. 25), so that changes in the metre definition could have more subtle implications than one might immediately expect. Certainly, despite the tight limits imposed on the constancy of the fine-structure constant by Turneaure and Stein²³, and by Schlayakhter²⁴ (on instantaneous and cosmological time scales respectively), it may be useful to monitor the ratio of the frequency of one of the lines of the Lyman, Balmer, or other series in atomic hydrogen with respect to that of the hydrogen maser — not only to test for the constancy of α , but also to ensure the coherence of our time and length scales for electromagnetic

radiation between the microwave and ultraviolet regions of the spectrum. No doubt tests of the constancy of the frequency of other highly stable sources in the visible region will provide further important auxiliary tests as well.

The above considerations suggest that we may have no unique 'best' way of realizing the unit of length (or time) in practical metrology. It is not impossible that in the future we might be able to maintain the metre more accurately, in terms of the wavelength of the radiation associated with an electron making the 1^2S-2^2S transition (by a two photon process in emission or absorption) in atomic hydrogen (or analogous transitions in a heavier atom, or ion), than it could be realized via the caesium-133 second and fixed velocity of light method. Laser cooling and trapped ion spectroscopy²⁶ promise impressive accuracies, $\sim 10^{-18}$, for both microwave frequency standards and visible and near-visible wavelength standards. Evidently, the continuance of the new definition would implicitly require that one could compare frequencies ranging from the microwave region through to the vacuum ultraviolet (VUV) and beyond, without loss of accuracy — otherwise we might have a microwave 'atomic second' and a VUV one, but this is part of the exciting metrology of tomorrow.

Realization

Subject to the adoption of the definition of the metre given in the introduction by the CGPM, the CCMD has proposed, in Recommendation M2 (1982), three distinct methods of realizing the metre. These are as follows (note that the definitive language is French):

(1) by means of the length l of the path travelled in vacuum by a plane electromagnetic wave in a time t ; this length is obtained from the measured time t , using the relation $l = ct$ and the value of the speed of light in vacuum $c = 299,792,458 \text{ m s}^{-1}$ exactly;

(2) by means of the wavelength in vacuum λ of a plane electromagnetic wave of frequency f ; this wavelength is obtained from the measured frequency f , using the relation $\lambda = c/f$ and the value of the speed of light in vacuum $c = 299,792,458 \text{ m s}^{-1}$ exactly;

(3) by means of one of the radiations given below, whose stated wavelength in vacuum, or whose stated frequency can be used with the uncertainty shown, provided that the given specifications and accepted good practice are followed; — and that in all cases any necessary corrections be applied to take account of actual conditions such as diffraction, gravitation, (note, relativity is effectively included by virtue of the reference to gravitation) or imperfection in the vacuum.

The specified radiations include: first, lasers stabilized by saturated absorption in specified conditions with reproducibilities

which are between 2 parts in 10^{10} and 2 parts in 10^9 ; second, radiations from spectral lamps, comprising specified transitions^{27,28} of ^{86}Kr , ^{198}Hg , and ^{114}Cd atoms with rather poorer reproducibilities — at the part in 10^8 or 10^7 level of precision.

All of the above realizations fall short of the accuracy with which the second may be realized at present, $<10^{-13}$, and they contain the necessary caveats that one would expect from the discussion in this note. The specified radiations will be modified as laser stabilization techniques improve and better absorption lines are found. It will be possible to alter them without modifying the actual definition of the metre and any changes will only concern those who need the unit of length to the highest accuracy achievable at any given time. It is expected that such changes will be discussed and agreed by the CCMD and CIPM, and documented in their proceedings as hitherto.

Conclusion

The definition of the metre is now moving to the final stage of endorsement and adoption by the CGPM in October 1983. The proposed definition has a deceptive simplicity and underlying beauty, and thereby achieves a neat solution to a number of physical problems, while providing adequately for the sophisticated needs of the science, technology, and education of today.

The views expressed here are personal ones. The author has enjoyed discussions with a number of colleagues, and those with Dr W.R.C. Rowley are particularly acknowledged. □

1. Giacomo, P. *Metrologia* **18**, 41-49 (1982).
2. Marx, B. *Nature* **296**, 110 (1982).
3. *Principles of Light Measurements* (Comite Internationale de l'Eclairage, Publ. no. 8, 1970).
4. Baldwin, G.C. *Phys. Rep.* **87**, 1-23 (1982).
5. Bay, Z. & White, J.A. *Phys. Rev. D* **5**, 796-806 (1972).
6. Goldhaber, A.S. & Nieto, M.N. *Rev. Mod. Phys.* **43**, 277-296 (1971).
7. Davis, L., Goldhaber, A.S. & Nieto, M.N. *Phys. Rev. Lett.* **35**, 1042-1045 (1975).
8. Primak, J.R. & Sher, M.A. *Nature* **268**, 680-681 (1980).
9. Dombey, N. *Nature* **288**, 643-644 (1980).
10. Dolan, L. & Jakiw, R. *Phys. Rev. D* **9**, 3320-3341 (1974).
11. Weinberg, S. *Phys. Rev. D* **9**, 3357-3378 (1974).
12. Linde, A.D. *Prog. teor. Phys.* **42**, 389-435 (1979).
13. Abbott, L.F. & Ciavola, M.B. *Nature* **299**, 187 (1982).
14. Woloshyn, R.M. *Phys. Rev. D* **27**, 1393-1395 (1983).
15. Sher, M.A. & Primak, J.R. *Nature* **299**, 187 (1982).
16. Vessot, R.F.C. *Radio Sci.* **14**, 629-647 (1979).
17. Flidrzynski, A. & Nowicki, A. *J. Phys. A* **15**, 1051-1059 (1982).
18. Kolen, P. & Torr, D.G. *Foundn Phys.* **12**, 401-410 (1981).
19. Bertotti, *Radio Sci.* **14**, 621-627 (1979).
20. McWhenny, E.W. *Nature* **243**, 196-200 (1973).
21. Dirac, P.M. *Nature* **139**, 323-327 (1935).
22. Petley, B.W. in *Quantum Metrology and Fundamental Constants* (eds Cutler, P.H. & Lucas, A.A.) (NATO ASI Ser., Plenum, New York, in the press).
23. Turneaure, J.P. & Stein, S.R. in *Atomic Masses and Fundamental Constants-3* (eds Sanders J.H. & Wapstra, A.H.) (Plenum, London, 1976).
24. Shlyakhter, A.I. *Nature* **264**, 340-346 (1976).
25. Beckenstein, J.K. *Phys. Rev. D* **25**, 1527-1535 (1982).
26. Dehmelt, H.G. *IEEE Trans. Instrum. Meas.* **IM-31**, 83-87 (1982).
27. CIPM (Procès-Verbaux CIPM, 49th session, 71-72, 1960; Comptes Rendus 11th CGPM, 85 (1960).
28. CCMD, 3rd session, 18-19 (1962); Procès-Verbaux CIPM, 52nd session, 26-27 (1963).

Planning more research on AIDS

The US Public Health Service has found an extra pot of gold for research on AIDS. Luckily, there is also now an agenda for making use of it.

It is natural enough that the US Public Health Service should have responded to public concern about acquired immune deficiency syndrome (AIDS) by throwing a modest amount of money at the problem (see page 365). The volume of correspondence (some of which appears on page 371) stimulated by a previous article on the subject (see *Nature* 28 April, p.749) is but one sign of how the unexpected appearance in the past few years of this bizarre disease has provoked a sense of nightmare. So how should the US Public Health Service spend its extra \$14.5 million?

Two features of what little is known of AIDS stand out. First, it occurs predominantly among male homosexuals and their sexual contacts of either sex, among certain ethnic groups (Haitian immigrants to the United States for example) and among haemophiliacs dependent on blood products for their survival. Second, because the root cause of the disease seems to be to render incompetent a class of cells (T-helper cells) essential for normal cell-mediated immunity, people with AIDS are usually recognized when they acquire some other disease, the otherwise unusual pneumonia *Pneumocystis carinii* caused by a protozoan organism or Kaposi's sarcoma, an otherwise uncommon skin cancer, for example. Because the T-cell population seems to be damaged irreversibly, mortality is high.

The nightmarish connotations of AIDS stem, however, not from the high mortality among those affected, for the incidence of the disease is not high even within the groups apparently susceptible to it, but from what has hitherto been sheer ignorance of the cause. Inevitably, in the past several months, people have been coming to the conclusion that there must be a transmissible agent and that the agent could well be a retrovirus (see *Nature* 28 April, p.749). One obvious model for such an agent has been the only virus so far known to be implicated in the transmission of malignancy, the retrovirus associated with the disease known as adult T-cell leukaemia or ATL (see *Nature* 14 April, p.567). The occurrence of some such virus was first recognized in south-west Japan, and it has now been fully characterized as HTLV (for human T-cell leukaemia virus) in Dr Robert Gallo's laboratory at the US National Cancer Institute. An important group of papers has now appeared (see *Science* 20 May) which confirms that HTLV is indeed somehow linked with

AIDS. Tantalizingly, however, these reports fall short of demonstrating that HTLV is the causative agent.

The new developments are nevertheless a significant pointer to the way in which the extra funds should be spent. Several suggestive and important pieces of information have come to light, largely as the result of work at the laboratories of Dr Max Essex at the Harvard School of Public Health and of Dr Robert Gallo at the National Cancer Institute, with the usual invaluable support of the Centers for Disease Control at Atlanta and with the collaboration of the Pasteur Institute in Paris. The most striking of the conclusions now reported are the following:

- Nineteen out of 75 male homosexuals suffering from AIDS have been found to carry antibodies against the surface antigens of a T-cell line infected with HTLV, as did six out of 23 patients with lymphadenopathy, thought to be a precursor form of AIDS (M. Essex *et al.*, *Science* 220, 859; 1983). By the same criteria, the occurrence of antibodies among matched control groups turned out to be numerically negligible but not entirely zero (one out of 81 matched male homosexuals).

- Two patients (both black) with AIDS have been shown to harbour in the DNA of their lymphocytes the now well characterized cellular version of the DNA sequence of HTLV (Edward P. Gellmann *et al.*, *Science* 202, 863; 1983). But the patients carrying the viral sequences were found only after an investigation of 33 patients suffering from AIDS. Mostly, HTLV could not be found.

- Both at the National Cancer Institute and the Pasteur Institute, it has been possible to isolate viruses which are either identical with or closely related to HTLV from the lymphocytes of patients (R.C. Gallo *et al.*, *Science* 202, 865; 1983 and F. Barre-Sinoussi *et al.*, *Science* 202, 868; 1983). In one case, the virus appears identical with one of the two known forms of HTLV; in the other (at Paris) the virus is closely related to HTLV but distinct from the forms so far characterized.

The significance of these developments is for the time being unclear, partly because the whole field is so new. But if the known virus were the causative agent of AIDS, it would be expected that antibodies would occur in all patients, not just a quarter of them. It is possible, of course, that the assay system, involving as it does novel

manipulations of T-cell systems, may be less than fully efficient, but that would be surprising. On this evidence alone, there is nothing to deny the alternative explanation that infection by HTLV is not so much the cause but a consequence of AIDS. But that also stretches credulity.

The other two findings are, for the time being, similarly equivocal. If HTLV were the cause of AIDS, would one not expect the viral genome to occur more frequently than in two out of 33 patients? Not necessarily, for if the precipitating defect of AIDS is a deficiency of helper T cells, there may be only a fleeting moment, during the early development of the syndrome, when there are enough cells carrying the integrated cellular form of the viral genome for detection to be possible. But the isolation of viruses (whose viral characteristics have been fully demonstrated by showing that they are indeed infectious) from the lymphocytes of two patients must strengthen the suspicion that there is a link of some kind between the virus and the syndrome. The French result also points to the likelihood that all the members of the HTLV family have not yet been found and to the possibility that in real life the constitution of the virus may be unusually labile.

These developments therefore suggest a host of ways in which a modest \$14.5 million might be spent. The search for antibodies among representative samples of the groups which are apparently susceptible to AIDS is plainly an urgent matter. So too is an investigation of links between viruses such as HTLV and two other groups or viruses — those, such as cytomegalovirus, which are thought to be implicated in Kaposi's sarcoma and those such as the cat leukaemia virus which similarly appear to affect the T-cell balance of the cat. No doubt this is where some of the extra funds will go.

It would be wrong, however, to expect that these investigations by themselves will be sufficient, at least while two other basic questions remain unanswered. First, there is a need for a fuller understanding of the consequences of retroviruses capable of causing malignancy for the biology of an infected cell. Second, the biology of T-cells is yet to be fully described but is fortunately now the centre of many people's interest. And one of the materials found in recent years to stimulate the growth of classes of lymphocytes may turn out to be a palliative if not a cure.

John Maddox

Molecular structure

Architectural design of spherical viruses

from Aaron Klug

MANY small simple 'spherical' viruses have their nucleic acid encased and protected in a closed shell or capsid made up of many identical protein molecules. This construction raises an interesting problem for a structure that must be capable of assembling itself without external instructions.

The simplest way to achieve spontaneous assembly is by specifying a structure with rotational symmetry, so that each unit is equivalently related to all the others, and all of them thus have identical bonding. The maximum number of units that can be so assembled is 60, and this arrangement has the symmetry of the icosahedron. It is thus perhaps not surprising that in all cases so far investigated, viral capsids do in fact have icosahedral symmetry. The difficulty is that most of them have many more than 60 subunits. To overcome this difficulty, Caspar and I proposed twenty years ago the notion of quasi-equivalence¹: in certain restricted arrangements, with icosahedral symmetry, much larger numbers of subunits could have nearly the same local environment and hence form nearly equivalent bonds to their neighbours, allowing self-assembly. The high-resolution X-ray crystallographic analysis of two small RNA plant viruses by the groups of Harrison² and Rossmann³ have shown substantial conservation of bonding specificity of the sort predicted by the quasi-equivalence theory.

This idea of quasi-equivalence as the basis for all the icosahedral designs predicted on the Caspar-Klug theory was, however, called into question a year ago by work from Caspar's laboratory at Brandeis University on the structure of the capsid of polyoma virus⁴, where the results suggest that the contacts between protein subunits are not all quasi-equivalent⁵. Many workers in the field⁶ have reserved final judgement on this until the study reaches higher resolution and is subjected to further checks. But in the meantime, the

Brandeis group has gone on to investigate a related topic — the structure of tubular variants of the polyoma virus — and in this issue of *Nature* present results which help to strengthen their earlier conclusions⁶.

If it turns out that quasi-equivalent bonding does not lie at the basis of the polyoma structure, what then does? For despite the Brandeis results, the arrangement of clustered subunits (capsomeres) found⁹ on the polyoma capsid does conform to one of the restricted icosahedral arrangements predicted by the Caspar-Klug theory. In this note, I shall give reasons for the general occurrence of these icosahedral arrangements and offer a particular explanation for the case of polyoma virus: possibly, as well as being selected for self-assembly, icosahedral structures may be dictated by the need for close-packing of the completed shell. But to develop this idea and indeed to explain the molecular significance of the new results on the polyoma tubes⁶, I need to outline the theory.

The Caspar-Klug theory is based on the solution to the problem of dividing the surface of a sphere into many identical, or near-identical, areas. Division into 20 equilateral triangles — that is into a regular icosahedron (see Fig. 1) — gives the maximum possible number of exactly equal subdivisions. If in a viral capsid three identical protein molecules make up each of the 20 triangles, there will be 60 exactly equivalent molecules in the shell — which, as I have mentioned, is the theoretical maximum number of asymmetric objects that can be arranged in identical environments in a closed shell. However, a further and finer, but now only approximately equal, division of the surface is obtained by effectively subdividing each face of the icosahedron into further (nearly) equilateral triangles. This produces triangulated surfaces having, like the icosahedron, 12 5-fold vertices but possessing additional 6-fold vertices. Each such icosahedral

lattice is classified by its so-called triangulation number (*T*-number), and the first four in the series are shown in Fig. 1.

All 'spherical' viruses so far investigated conform to one of these lattices. Thus the polyoma virus, like the papilloma viruses⁷⁻⁹, belongs to the class $T=7$ and is built of 72 capsomeres, 12 located at the 5-vertices and 60 at the 6-vertices (see Fig. 1 legend). However, if each triangular facet were, as the quasi-equivalence theory demands, associated with three identical protein molecules, then the capsomeres located at the 6-vertices, where six triangular facets meet, would correspond to a hexamer and similarly those at 5-vertices would correspond to pentamers. The original results from Brandeis contradicted this, and all capsomeres were proposed to be pentamers⁴. This would mean that the bonding between coat protein subunits is distinctly non-equivalent and indeed three types of contact between neighbouring pentamers exist, a conclusion which is not pleasing aesthetically and would seem difficult to implement in molecular terms.

At this point, we can return to the paper in this week's *Nature*⁶. It concerns the structure of abortively assembled viral capsids of the papilloma-polyoma family that instead of being spherical are hollow tubular particles with rounded ends, containing little or no DNA. They seem to be assembled from the same major protein component as the normal shell, but with the capsomeres arranged over the surface of a cylinder instead of a sphere. Finch and I⁸ originally noted that there were two main types of these tubular variants: wide tubes of diameter about 500 Å and narrower tubes of diameter about 300 Å. The wide type were called 'hexamer' tubes because they are built of approximately hexagonally coordinated capsomeres, that is each has six close neighbours (compare tubular variants of bacteriophage¹⁰). The capso-

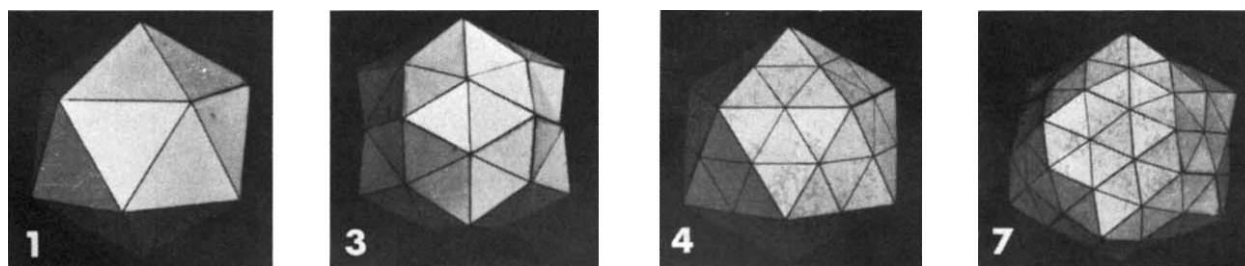


Fig. 1 Triangulated surfaces representing the first few icosahedral lattices, with their *T*-numbers marked below. $T=1$ gives the basic icosahedron. The lattice relevant to polyoma virus is $T=7$: it has 60 6-vertices and 12 5-vertices. The total number of triangular facets is $20T$, and if we assume, as before, that three protein molecules make up each facet, there would be $60T$ protein molecules in all, which will be quasi-equivalently related to each other. The possible values of the triangulation number T are given by the formula $T = H^2 + HK + K^2$, where H and K are positive integers; this produces the series $T = 1, 3, 4, 7, 9, 12, \dots$. The icosahedral surface lattice has $10(T-1)$ 6-fold vertices, and 12 5-fold vertices, making $10T+2$ vertices in all.

meres in the narrow tubes were arrayed in a novel 'pentagonal tessellation' which arises from the contact of pentamers across the 2-fold axes of a particular lattice¹¹. It thus appeared in 1969 that the protein subunits could aggregate into both hexamers and pentamers, as required by the quasi-equivalence theory.

The new paper by Baker *et al.*⁶ now demonstrates that the so-called hexamer tubes are also composed of pentamers. They re-examined the wide tubes using low-dose electron microscopy, which gives much better preservation and, combined with image processing, shows small but significant departures from hexagonal packing and resolves the capsomeres into pentamers. (These pair in a similar manner to those found by Kiselev and Klug in the narrow tubes¹¹, but the packing of pairs is different.) It thus appears that both classes of tube are polymorphic aggregates of the capsomeres, and strengthens the view that the capsomeres of the virus shell itself are also pentamers, as proposed by Rayment *et al.*⁴.

If this is confirmed by high-resolution X-ray analysis, then one needs to re-think the reasons for the occurrence of icosahedral symmetry in spherical viruses, and in particular for the fact that all the spherical viruses so far observed have gross structures corresponding to one of the icosahedral lattices enumerated by Caspar and Klug. In our original paper¹ we noted the difficulties that might be encountered in building shells with large T -numbers out of identical units, and evidence has accumulated that many of the larger viruses (for example bacteriophage T4 and adenovirus) are built of more than one kind of protein. One of the advantages of icosahedral symmetry was pointed out a long time ago by Finch and myself¹², namely that it allows the use of the greatest number (60) of asymmetric units to build a framework, so it is economic of a given type of protein molecule.

We also pointed out that if one desired to 'enclose' a space around a central point by a set of domains on a closed surface, the ratio of the number of domains to the surface area covered, or the volume enclosed, is smallest in the case of icosahedral symmetry (see also ref. 1). This can be seen from the manner of derivation of the icosahedral surface lattices described above. In a plane the densest packing of units is achieved if they are arranged in a hexagonal lattice in which each unit has six nearest neighbours, that is they lie at the vertices of a triangular mesh, consisting of 6-vertices. This is topologically impossible in a sphere, where curvature must be introduced. To maintain this close packing as far as possible when the plane surface is deformed into a closed surface, what is required topologically is that 12 of the 6-vertices be turned into 5-vertices, and, for as nearly a uniform covering of the sphere as possible, these 5-vertices must be spaced as far apart from each other as possible, that is at the vertices of an icosahedron.

Hence we arrive at the same icosahedral lattices as found in the Caspar-Klug theory, but the reason is now a geometrical one (economy of covering) rather than a structural one (property of quasi-equivalence).

More rigorously, it can be shown by adapting some of the geometrical theorems in, say, the book by Toth¹³ that asymmetric units placed at the vertices of the icosahedral lattices represent particularly economical packings of points placed on the surface of a sphere. The mathematical question is the so-called Tammes problem of maximizing the least distance between a given number of points placed on the surface of a sphere. The further the points are

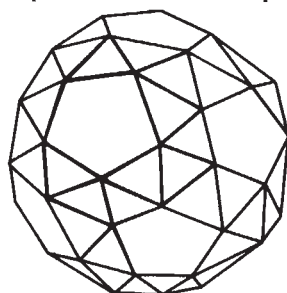


Fig. 2 The snub dodecahedron, an Archimedean polyhedron with strict icosahedral symmetry. Its 60 vertices are arranged as are the 6-vertices in the $T=7$ lattice of Fig. 1, and represent the densest arrangement of 60 equivalent points on a spherical surface.

apart the greater the area covered by a domain associated with each one. (Mathematically, some marginally more favourable packings can be found in some cases if the icosahedral symmetry is reduced to 5-fold axial symmetry¹⁴, but then the advantage in economy of using the maximum number of structural units of one type — that is 60 with icosahedral symmetry — would be lost.) The icosahedral surface lattices therefore emerge as the optimal way of arranging units in a close-packed array on a closed surface. Close-packing allows groups of protein molecules to form a tight shell which shuts out the outside world from nucleic acid in the virus interior.

On this idea one must distinguish between the geometrical design of a shell and its structural realization. Shells with small T -numbers can certainly be built out of identical units using quasi-equivalent bonding^{2,3}. Larger shells might entail a different mode of construction, with specialized units in different places on the icosahedral lattice. An example of the latter is to be found in adenovirus¹⁵, and also in the case of the icosahedral capsid of bacteriophage where it can be seen using electron microscopy¹⁶ that the capsomeres at the 5-vertices have a different appearance from those at the 6-vertices, and are chemically distinct¹⁷.

Where the borderline lies is a matter for experiment. If the results on polyoma virus hold up, how then can one envisage the construction of the shell? One would imagine that 60 units, in this case pentamers,

are placed at the 60 6-vertices of the $T=7$ lattice, and these are exactly equivalently related by icosahedral symmetry and so could self-assemble perfectly, even though all individual monomers are not in identical environments, a condition not infrequent in protein crystals. In fact these 60 units lie at the vertices of a snub dodecahedron (Fig. 2), which represents the densest arrangement of units with perfect icosahedral symmetry $T=1$. This arrangement would leave 12 holes on the 5-fold axes, and these could be filled by a second set of 12 pentamers, which would make a different type of contact with the first set. The packing of the pentamers in the two types of tube may reflect the two types of contact found in the virus shell. It remains to be seen whether this 'variable bonding potential', as Baker *et al.*⁶ call it, is a property of a single multifunctional protein molecule (the subunit of the pentamer) or whether this is perhaps brought about by some modification of one of the two sets of capsomeres. Thus it should be remembered that besides the major protein component of the polyoma virus shell, VPI, there are two minor components present forming at least 10 per cent of the total virion protein¹⁸, whose role is not known. One might imagine that these could become associated with the 12 pentamers on the 5-vertex and so modify their bonding potential. Alternatively the latter could be chemically modified before virus assembly. In any case, a better understanding of the chemistry is needed.

Whatever the answer, the Brandeis group has opened up a fascinating and important structural problem, and one which reminds us that knowledge of the macroscopic design of a structure does not automatically tell us how it is realized at the molecular level. Their work shows that the quasi-equivalence theory does not provide a sufficient explanation of the structure of all spherical viruses. The theory was never claimed to be exclusive, but the underlying geometrical idea used in that theory, thought of in a more general way, may help us to understand why icosahedral designs have been selected. □

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1. Caspar, D.L.D. & Klug, A. *Cold Spring Harb. Symp. quant. Biol.* 27, 1 (1962).
2. Harrison, S.C. *et al. Nature* 276, 368 (1978).
3. Abad-Zapatero, C. *et al. Nature* 286, 33 (1980).
4. Rayment, I., *et al. Nature* 295, 110 (1982).
5. See Eisenberg, D. *Nature* 295, 99 (1982).
6. Baker, T., *et al. Nature* 303, 446 (1983).
7. Klug, A. & Finch, J.T. *J. molec. Biol.* 11, 403 (1965).
8. Finch, J.T. & Klug, A. *J. molec. Biol.* 13, 1 (1965).
9. Finch, J.T. *Gen. Virol.* 24, 359 (1974).
10. DeRosier, D.J. & Klug, A. *J. molec. Biol.* 65, 469 (1972).
11. Kiselev, N.A. & Klug, A. *J. molec. Biol.* 40, 155 (1969).
12. Finch, J.T. & Klug, A. *Nature* 183, 1709 (1959).
13. Toth, L.F. *Lagerungen in der Ebene, auf der Kugel und in dem Raum* (Springer, Berlin, 1953).
14. Goldberg, M. *J. molec. Biol.* 24, 337 (1967).
15. Ginsberg, H.L. *Comprehensive Virology* Vol. 13 (eds Fraenkel-Conrat, H. & Wagner, R.R.) Ch. 6 (Plenum, New York, 1979).
16. Branton, D. & Klug, A. *J. molec. Biol.* 92, 559 (1975).
17. Müller-Salamin, L., *et al. J. Virol.* 24, 121 (1977).
18. Tooz, J. in *DNA Tumour Viruses* (ed. Tooz, J.) (Cold Spring Harbor, New York, 1980).

RNA processing

Lessons from mutant globins

from Stephen Mount and Joan Steitz

THE human thalassaemias, which are genetic diseases characterized by defective synthesis of the polypeptide chains of haemoglobin, have provided a remarkable illustration of the versatility of gene expression in mammalian cells. Many thalassaemias¹⁻⁸ are due to mutations that interfere with the correct removal of intervening sequences (introns) from globin transcripts, and can therefore be categorized as splicing defects. Recent analyses of these thalassaemias, as well as the deliberate construction of mutations affecting the splicing of globin transcripts^{9,10}, indicate how the complexity of the splicing mechanism might contribute not only to accident-proneness, but also versatility, to protein synthesis. They also pose the question of how to reconcile the aberrant splicing patterns observed with current models for the normal splicing mechanism.

The first clue to the question of how introns might be removed from the transcripts of split genes came from the observation that short sequences at both ends of introns are highly conserved. These conserved sequences (especially those at 5' splice sites) exhibit significant complementarity to the 5' end of U1 RNA, and it has therefore been suggested that the small nuclear RNA-protein complex that contains U1 is the cellular component responsible for recognizing the ends of introns^{11,12}. However, it is easy to find sequences that agree with the 5' consensus but are not used as splice sites, suggesting that while complementarity to U1 may be necessary for splicing it cannot be sufficient to determine exactly where splicing will occur. This situation is analogous to that documented for the recognition of translation initiation sites on mRNA by bacterial ribosomes; not all mRNA regions complementary to 16S rRNA, even when appropriately positioned before an initiation codon, do in fact operate as start sites.

The thalassaemia mutations, and other naturally occurring¹³, induced¹⁴, or constructed^{9,15-17} splice-site mutants, confirm the importance of conserved nucleotides to the function of 5' splice sites. Use of a 5' splice site is abolished by all mutations

within the nearly invariant G-T dinucleotide located precisely at the 5' end of an intron and is decreased by a number of mutations elsewhere in the nine-nucleotide consensus region. The surprising result, obtained both with the thalassaemias^{1,5,6} and with the rabbit β -globin gene mutated *in vitro*⁹, is that inactivation of a 5' splice site does not simply prevent splicing. Instead, the 3' splice site is joined to other sequences which match the 5' splice-site consensus but are not normally used. In the case of the human β -globin small intron, there are three such cryptic sites and all three are used in each of three different 5' splice-site mutants analysed by Treisman *et al.*¹. Interestingly, one of these alternative sites (located in the preceding exon) is also activated by at least two separate mutations that make it more closely resemble the 5' splice-site consensus^{7,18}.

Two single-nucleotide changes which create new 5' splice sites within the 850-nucleotide second intron of human β -globin uncover a cryptic 3' splice site upstream in the same intron (nucleotide 579). A mutation creating a 5' splice site at nucleotide 745 leads to a low level of normally spliced RNA and a large amount of RNA with an extra exon formed from nucleotides 580-744 (splices remove nucleotides 1-579 and 745-850; see the figure)¹. Mutation at nucleotide 705 leads to a small amount of RNA with an extra exon (nucleotides 580-705), no normally spliced RNA, and a predominant RNA in which the normal 5' splice site is joined to the cryptic 3' splice site at nucleotide 579 but nucleotides 706-850 are retained⁸. Thus, the generation of a 5' splice site within this intron interferes with the proper splicing even of transcripts in which the new 5' splice site is not used.

In a more recent paper, Wieringa *et al.*¹⁰ explore similar phenomena by constructing rabbit β -globin gene variants in which fragments containing 5' or 3' splice sites are placed upstream or downstream of their natural counterparts. In each construct splicing took place between the outermost sites. These results all serve to discredit simple scanning models in which the splicing

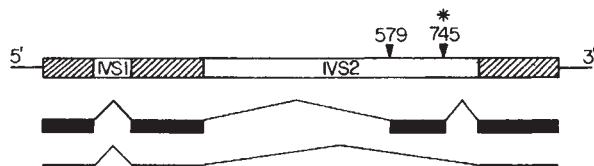
machinery first associates with one splice site and moves one-dimensionally in search of a counterpart. As Wieringa *et al.* argue, more complex models, particularly those which take into account the conformation of the splicing substrate, including the secondary and tertiary structure of the RNA and the presence or absence of RNA-binding proteins, seem necessary.

At one of the cryptic 5' splice sites in the rabbit β -globin gene⁹, splicing occurs adjacent to G-C rather than G-T, previously considered to be the single invariant feature of the 5' consensus sequence. Two natural 5' splice sites containing G-C in this position have only recently been reported. One is an infrequently used 5' splice site associated with an optional exon in murine α A-crystallin¹⁹, and the other is a chicken α -globin 5' splice site²⁰. Splicing 5' to G-C in these three cases demonstrates that G-C can serve as an occasional alternative to G-T, much as the initiation of protein synthesis in bacteria sometimes occurs at GUG rather than AUG codons.

Whereas splicing at most cryptic 5' splice sites takes place at the normal location within a consensus region, splices at two cryptic 5' splice sites in the mutated rabbit β -globin gene occur at an unusual point within the 5' splice-site consensus region⁹:

5' Consensus:	C	A	G	T	A	G	T
	A	A	G	T	G	A	G
Cryptic 1:	A	G	G	A	T	G	A
Cryptic 2:	A	A	G	C	T	G	A

One possible explanation for the displacement of the splice point stems from the proposal that U1 snRNA-protein complexes recognize 5' splice sites by base-pairing^{11,12}. A match with the consensus may reflect one requirement of the splicing mechanism (the need to form a RNA-RNA double helix with U1 snRNA), and the conservation of G-T another (the specificity of a nuclease or nick-ligase). The use of these cryptic splice sites makes sense if they are secondary binding sites for U1 snRNPs and if the cutting activity that usually requires G-T is not absolutely constrained to act at its normal position within the intermolecular helix.



Processing of second-intron (IVS2) position 745 mutant transcripts. The β -globin gene is shown, with exons represented by hatched boxes and intervening sequences by open boxes; flanking sequences are shown as thin lines. The locations of the mutation (asterisk) and the cryptic 3' splice site at IVS2 nucleotide 579 are arrowed. Below are shown the two different RNAs produced by the gene in cultured cells: the exons are shown as solid blocks, with splices indicated by carets.

1. Treisman, R. *et al.* *Nature* **302**, 591 (1983).
2. Busslinger, M. *et al.* *Cell* **27**, 289 (1981).
3. Fukumaki, Y. *et al.* *Cell* **28**, 585 (1982).
4. Orkin, S.H. *et al.* *Nature* **296**, 627 (1982).
5. Felber, B.K. *et al.* *Cell* **29**, 895 (1982).
6. Treisman, R. *et al.* *Cell* **29**, 903 (1982).
7. Orkin, S.H. *et al.* *Nature* **300**, 768 (1982).
8. Dobkin, C. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **80**, 1184 (1983).
9. Wieringa, B. *et al.* *Nature* **301**, 38 (1983).
10. Wieringa, B. *et al.* *EMBO J.* **2**, 727 (1983).
11. Lerner, M.R. *et al.* *Nature* **283**, 220 (1980).
12. Rogers, J. & Wall, R. *Proc. natn. Acad. Sci. U.S.A.* **77**, 1877 (1980).
13. Esumi, H. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **80**, (1983).
14. Benyajati, C. *et al.* *Nucleic Acids Res.* **10**, 7261 (1982).
15. Solnick, D. *Nature* **291**, 508 (1981).
16. Montell, C. *et al.* *Nature* **295**, 380 (1982).
17. Gallwitz, D. *Proc. natn. Acad. Sci. U.S.A.* **79**, 3493 (1982).
18. Goldsmith, M.E. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **80**, 2318 (1983).
19. King, C.R. & Piatigorsky, J. *Cell* **32**, 707 (1983).
20. Dodgson, J.B. & Engel, J.D. *J. biol. Chem.* **258**, 4623 (1983).
21. Gilbert, W. *Nature* **271**, 501 (1978).
22. Langford, C. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **80**, 1496 (1983).

Thus, while data from mutations that affect splicing confirm the idea that the sequences of splice sites are crucial to their ability to function, they also raise important questions about the complex mechanism that governs whether or not a particular splice site will be used and with which partner it will be coupled. Such complexity reflects the extreme versatility of messenger RNA splicing in higher eukaryotes, which in turn may contribute to the evolution of new proteins²¹. The use of a cryptic splice site allows the testing of a new protein, often without altogether giving up the old one^{1,7,18}.

In the thalassaemias none of the new arrangements is at all satisfactory, but it is easy to see how the optional exon in αA -

crystallin and similar polymorphisms in other proteins could have arisen from aberrant splices like those discussed here. Gallwitz and his colleagues have shown both that mutations in the 5' splice site of the yeast actin gene do not give rise to cryptic splices¹⁷ and that yeast is unable to remove vertebrate introns from hybrid genes²². The adaptability of the splicing machinery demonstrated by the thalassaemia mutations may therefore be restricted to higher eukaryotes, and genetic defects such as these may be a price we pay for the evolutionary advantages we enjoy. □

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as at 20°C. Just a few more should provide the same stability at 250°C. It seems quite possible therefore that these newly discovered extreme thermophiles may have proteins not fundamentally different from the norm, though Baross and Deming do report that while all the usual protein amino acids are present, there are in addition five of unknown identity.

The new bacteria, like other extreme thermophiles, are probably archaeobacteria, organisms which, with a long separate evolution from the more cosmopolitan eubacteria, have membranes based on a different design — long-chain ether lipids^{5,6} that span from one surface to the other. They form thermostable structures.

Can the DNA of these bacteria be kept in its double helix? Unless it can, it would be difficult to synchronize replication of the complementary strands and impossible to repair them. Perhaps there are special proteins that maintain the duplex confirmation. It is easy to speculate on how the building blocks of life can be made thermostable, of course; the great achievement of Baross and colleagues has been to recognize that bacteria were growing at such exceptional temperatures when the conventional wisdom must have been shouting that it was impossible.

Their finding also has several important implications. The bacteria must produce highly thermostable enzymes and some may have application in laundry or other industrial processes involving high temperatures. They might also provide the basis of a new natural gas industry, as Baross and Deming have shown that these organisms generate hydrogen, carbon monoxide and especially methane during growth. For pure science, though, there is also substance for speculation on the origin of life. While I do not concur with the idea⁷ that a single, extremely unlikely, chance event was required to set life in motion, it is clear that the time required for evolution of chemical conglomerations into living forms would be much less at 250°C than at the ambient temperature. Moreover, life could have originated much earlier in the development of the cooling Earth than has been previously considered. And what lies below those submarine cisterns spewing forth their bacterial brew — other forms of life, perhaps? My experience with another bizarre life form, the square bacterium⁸, is that once you have discovered the first one others are not then so difficult to find. □

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Microbiology

Bacteria that grow at 250°C

from A.E. Walsby

THE title of Ray Bradbury's science fiction novel *Fahrenheit 451* is a reference to the temperature at which paper ignites. Almost stranger than fiction is the report in this issue of *Nature* by J.A. Baross and J.W. Deming¹ that there are forms of life capable of growing at even higher temperatures, 250°C (482°F) or more. This astounding discovery not only challenges our preconceptions about the limits at which organic life can survive, it also alerts us to the possibility that life may exist within regions of the Earth's crust (and perhaps on other planets) previously presumed sterile on account of their elevated temperatures. Perhaps life may even have originated in those superheated systems.

The paper by Baross and Deming is a sequel to that by Baross, Lilley and Gordon² which describes complex communities of bacteria in hot water rising from sulphide-encrusted vents located along tectonic rifts and ridges on the deep ocean floor. Some of these communities occurred at temperatures exceeding 360°C and from them were isolated bacteria that could grow in the laboratory at 100°C, obligate thermophiles whose growth ceased below 75°C. Baross *et al.* speculated that these bacterial communities might be the source of much methane, hydrogen and carbon monoxide that vents into the ocean.

Baross and Deming have now grown some of these bacteria in enriched seawater enclosed in a titanium syringe surrounded by a chamber heated to 250°C and pressurized at 265 atmospheres, the hydrostatic pressure at the depth (2,500 m) of the ocean floor where the bacteria were found. At this pressure seawater does not boil until it reaches 460°C. Several different bacteria grew in this pressure-cooker arrangement, doubling in number in about 40 minutes — the same sort of growth rate commonly encountered in many mesophilic bacteria at ambient temperatures. The evidence for

growth at 250°C was very clear cut: the number of bacteria increased a hundred-fold over a few hours, as did the protein they contained. There are indications that the bacteria will grow with similar vigour at 300°C though this has yet to be confirmed. And even this may not be the upper limit.

If only 250°C should transpire to be the maximum for growth this sets a new record by a large margin: until now the highest temperature for a thermophile, also from a hot submarine floor, was 105°C (ref. 3). The latest discovery amply confirms Brock's postulate that "certain kinds of bacteria may perhaps be able to live in any boiling habitat that contains liquid water" and his observation that "the upper temperature for life has not yet been defined"⁴.

I must admit that my first reaction on reading the manuscript of Baross and Deming, arriving as it did on the eve of April Fool's Day, was one of incredulity. Are we not, after all, conditioned to believe that most germs will succumb after Pasteurization at a mere 70°C, and that just 20 minutes at 120°C in the autoclave will exterminate the most resistant bacterial spore? Have we not also learned in biochemistry classes that at these scalding temperatures proteins denature, lipids run to ghee and duplex DNA melts into separate strands?

Some of the same questions have been asked before, of course, in relation to thermophilic bacteria growing at up to 100°C. But will the same explanations hold at 250°C? To take the case of proteins, for example, it is known that they are not all denatured like albumen in the five-minute egg, and not all enzymes, even from mesophiles, are inactivated by boiling, the biochemist's routine control. It can be argued from the thermodynamics of protein denaturation that only a few extra hydrogen bonds or salt bridges are needed to provide the same stability in a protein at 100°C

1. Baross, J.A. & Deming, J.W. *Nature* **302**, 423 (1983).
2. Baross, J.A. *et al.* *Nature* **298**, 366 (1982).
3. Stetter, K.O. *Nature* **300**, 258 (1982).
4. Brock, T.D. *Thermophilic Microorganisms and Life at High Temperatures* (Springer, New York, 1978).
5. De Rosa, M. *et al.* *Phytochemistry* **15**, 143 (1975).
6. Langworthy, T.A. *Biochim. biophys. Acta* **389**, 477 (1977).
7. Hoyle, F. & Wickramasinghe, C. *Evolution from Space* (Dent, London, 1981).
8. Walsby, A.E. *Nature* **283**, 69 (1980).

Astronomical achievements

The best is yet to be

from Virginia Trimble

WE are all more or less conditioned to believe that scientists are most productive very early in their careers. This has undoubtedly been true for many, including some, such as Newton and Einstein, whose contributions were truly revolutionary. But it is not the whole story, at least for outstanding (though non-revolutionary) twentieth-century American astronomers. Helmut Abt (editor of *Astrophysical Journal*) has charted out^{1,2} the publication histories of 115 people who received doctorates in astronomy in the USA between 1945 and 1960, and the citation histories of papers written at various ages by 22 very productive astronomers whose careers were largely complete before 1970. Both studies yield surprises.

The 1945–1960 cohort, apart from moderate peaks associated with the award of doctorates and the 'tenure crunch' 5–8 years later, have continued to produce research papers in refereed journals at a nearly constant rate over the next 25 years — despite an immediate postdoctoral loss of 9 per cent of the group from the ranks of the published and an annual 1.5 per cent loss thereafter. Thus, the more active members of the cohort show publication records that increase monotonically with time, until, 20–25 years postdoctorally, the 13 most productive are accounting for 65 per cent of the published pages. The members of this group have now reached career stages at which international scientific reputation and participation are likely to peak. (The only journals scanned were *Astrophysical Journal*, including *Letters* and *Supplements*, *Astronomical Journal* and *Publications of the Astronomical Society of the Pacific*, the three American periodicals that publish original research papers over the full range of astronomy and astrophysics. Papers were normalized to 1,000-word pages divided by the number of authors of the paper. Inclusion of non-American journals, conference proceedings and review publications in the data base would probably have resulted in a still steeper rise with time in published pages for the more prolific authors.)

Contrary to other cherished prejudices, the high-productivity group (all are men) are not preferentially employed by full-time research institutions and are not much over-represented in the National Academy of Sciences, relative to other members of their age group. Several are widely known for their willingness to take on committee, conference and administrative responsibilities; others are rarely seen outside their own offices. Abt concludes that productive astronomers are largely self-motivated and manage to function well regardless of environmental pressures.

Even less expected is the record of paper citations, which provides some measure of how influential individual authors have been at various times (though it is possible to be too influential; most papers on Hertzprung–Russell diagrams do not cite either Hertzprung or Russell). For this study, astronomers were selected as having published frequently in *Astrophysical Journal* before 1970; their work was therefore largely done in the USA, but most received their degrees before 1945 and there is little overlap with the previous group. Data came from *Science Citation Index* and so reflect papers from a very wide range of periodicals and books, for which the individuals investigated were senior or sole authors.

The first surprise is the sheer number of citations — 9,435 (from 1970 to 1979) to nearly 1,000 papers (published from before 1910 to after 1970; median 1951) by 21 men and 1 woman (130–1,400 citations each; mean 430). The second is that half of all citations are to papers published by authors 58 years old or older, and this drops only to about 53 when correction is made for the exponential fall (half life 24 yr)^{2,3} in citation rate as papers age past their peak influence,

achieved 5 years after publication.

Of the 22 authors, four wrote their most-cited papers in their 70s, six in their 60s, four in their 50s, five in their 40s, one in his 30s, none in their 20s and two were bimodal (30s + 50s and 30s + 60s). Many show a relative minimum in middle life, representing the war years when their attentions were wholly or partly directed away from astronomical research. At least four of the cohort continued to publish after 1970 and piled up a few dozen more citations to these late papers during 1980 and 1981, though in no case enough to affect the total statistics. (I am indebted to H.A. Abt for additional information about the papers and individuals discussed in refs 1 and 2.)

What are we to make of all this? First, astronomers apparently live a long time (all 13 most productive of the 1945–60 cohort are still hard at it; 5 of the 22 earlier astronomers garnered 82 citations to papers written past age 80). Second, we can apparently expect to go on producing work that our colleagues will consider worth publishing and referencing for as long as we remain motivated to try. And, for most of us, if not our best work, at least our most cited work is probably still ahead of us. □

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1. Abt, H.A. *Publs astr. Soc. Pacif.* **94**, 213 (1982).
2. Abt, H.A. *Publs astr. Soc. Pacif.* **95**, 113 (1983).
3. Abt, H.A. *Publs astr. Soc. Pacif.* **93**, 207 (1981).

Immunology

Graft rejection and Ia antigens — paradox resolved?

from A. Neil Barclay and Don W. Mason

AN apparent paradox arising from experiments on the rejection of skin allografts between mouse strains incompatible for Ia antigens may be resolved by results reported in a paper published in this issue of *Nature*¹.

Ia antigens are highly polymorphic membrane glycoproteins coded for by genes of the I region of the mouse major histocompatibility complex (MHC)². Homologous glycoproteins have been found in all mammalian species and are collectively termed class II antigens irrespective of the species involved. The importance of these antigens in immunology lies in the fact that T cells with helper/inducer activity recognize foreign antigen only when it is presented in association with class II glycoproteins expressed on antigen-presenting cells (APCs). In addition, the class II glycoproteins are themselves potent transplantation antigens as evidenced by their unique ability to stimulate a very high frequency of allogeneic T cells *in vitro* and to cause rejection of class II-incompatible grafts *in vivo*. This high level of alloreactivity induced by class II antigens is of prac-

tical importance in organ transplantation in man, as matching of donor and recipient for class II antigens is associated with an improved chance of graft survival³.

The paradox arises because class II antigens have a restricted tissue distribution, unlike class I antigens (the so-called classical transplantation antigens) which are present on the cells of most tissues. In normal mouse skin, for example, the only class II antigen-positive cells are the dendritic cells of the dermis and the Langerhans cells of the epidermis, both of which are bone-marrow-derived. Thus it has been assumed that when a mouse of one strain rejects a skin graft from a mouse of another strain differing only in its class II antigens, the graft is destroyed because of the reaction of the host to the bone-marrow-derived cells in the graft.

However, experiments of a differing type have shown that this hypothesis is incorrect. These experiments involved the use of donor mice of the same strain as the recipient, but whose bone marrow had been destroyed by irradiation and replaced with class II-incompatible bone marrow.

Skin grafts from these radiation chimaeras should be rejected by virtue of their incompatible dendritic and Langerhans cells, but they are not⁴. This implies that class II antigens on these bone-marrow-derived cells are not a sufficient stimulus for graft rejection. What, then, can account for the rejection of skin grafts between mouse strains differing only in their class II antigens?

De Waal *et al.* have provided a solution to this paradox by showing that in such skin grafts, the expression of class II antigens is induced on the non-bone-marrow-derived cells of the vascular endothelium. They suggest that this happens because the allogeneic Langerhans cells in the grafts activate the host T cells, and they in turn induce class II antigen expression on the vascular endothelium. Thus it is the vascular endothelium that serves as the target for the alloaggressive cells of the host. (The same sequence of events may occur in mice with skin grafts from donors that are radiation chimaeras and in which only the bone-marrow-derived cells are class-II-different; but in these grafts, the vascular endothelium is class-II-identical to the host, so no rejection ensues.)

This is not the first report of induced expression of class II antigens: they can be induced by activated T cells *in vivo* on macrophages, epithelial cells of intestinal crypts and on keratinocytes, and soluble T-cell products have been shown to induce them *in vitro* on macrophages and vascular endothelium⁵⁻⁷. Furthermore they may be induced by non-T-cell stimuli: in the epithelium of the lactating mammary gland, class II expression is under hormonal control⁸. Of more relevance in the present context is the observation that class II antigens can also be induced on the keratinocytes of rat skin allografts⁹.

Whether the findings of de Waal *et al.* have any significance in human organ transplantation is uncertain. It has been shown that the endothelial cells of human umbilical vein can be induced to express class II antigen under the influence of activated T cells so inducibility is not a phenomenon confined to rodents⁷. On the other hand, the renal and cardiac vascular endothelia of man, unlike those of laboratory rodents, seem to express class II antigens constitutively^{10,11}.

There is no known role for the class II antigens that can be induced on the various cell types that do not express them normally. Three types of function could be envisaged. (1) The antigens are involved in interactions unrelated to the immune system. This seems unlikely in view of the finding that immunological stimuli can induce their expression and the molecules are known, at least in some circumstances, to be involved in antigen recognition by T cells.

(2) The molecules present antigen to T cells and this is their only role. Epithelium of the gut and epidermal keratinocytes are exposed to environmental antigens and are accessible to T cells so this hypothesis is not

unattractive for these tissues. It is perhaps less obvious how the expression of class II antigens on vascular endothelium fits in with this suggestion but pathogenic organisms and their toxins do gain access to the bloodstream.

(3) Class II antigens are involved in aspects of the immune system other than antigen presentation, for example in the organization or recruitment of T lymphocytes. The fact that epithelium of lactating, but not inactive, mammary gland of the guinea pig expresses class II antigens may best be explained in this way⁸.

In attempting to decide which, if any, of these suggestions is correct it should be noted that the presentation of antigen to a T cell by an APC is probably not a passive process but may involve interleukin production by the APC¹². It is therefore significant that cultured vascular endothelium expressing class II antigens can induce cell proliferation of allogeneic T

cells¹³ and keratinocytes produce a factor resembling interleukin-1 (ref. 14). □

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1. de Waal, R.M.W. *et al.* *Nature* **303**, 426 (1983).
2. Klein, J. *et al.* *Nature* **291**, 455 (1981).
3. Morris, P.J. & Ting, A. *Immun. Rev.* **66**, 103 (1982).
4. Woodward, J.G., Shigekawa, B.L. & Frelinger, J.A. *Transplantation* **33**, 254 (1982).
5. Barclay, A.N. & Mason, D.W. *J. exp. Med.* **156**, 1665 (1982).
6. Steinman, R.M. *et al.* *J. exp. Med.* **152**, 1248 (1980).
7. Pober, J.S. *et al.* *J. exp. Med.* **157**, 1339 (1983).
8. Klareskog, L., Forsum, U. & Peterson, P.A. *Eur. J. Immun.* **10**, 958 (1980).
9. Ballman, M.J. & Mason, D.W. *Transplantation* (in the press).
10. Fuggle, S.V. *et al.* *Transplantation* **35**, 385 (1983).
11. Daar, A.S. *et al.* *Transplant Proc.* **15**, 311 (1983).
12. Röllinoff, M., Pfizenmaier, K. & Wagner, H. *Eur. J. Immun.* **12**, 337 (1982).
13. Hirschberg, H., Braathen, L.R. & Thorsby, E. *Immun. Rev.* **66**, 57 (1982).
14. Luger, T.A. *et al.* *J. Immun.* **128**, 2147 (1982).

Oncogenic intelligence

Evidence of a hit-and-run tumour virus

from Peter Newmark

In a provocative interpretation of some rather complicated data published in the 20 May issue of *Science* (**220**, 795; 1983), J. F. Mushinski, M. Potter, S. R. Bauer and E. P. Reddy suggest that the Abelson murine leukaemia virus sometimes becomes integrated into the cellular DNA of a precursor to a B lymphocyte just long enough to cause rearrangement of the cell's *c-myb* proto-oncogene. As a result, a new product is made from the rearranged *c-myb* and a plasmacytoid lymphosarcoma develops from the cell.

The facts are these. Five out of six plasmacytoid lymphosarcomas contained neither DNA nor RNA related to Abelson murine leukaemia virus (the exception proved to be very useful — see below). All six showed evidence of a rearrangement of their *c-myb* gene, the cellular homologue and presumed progenitor of the transforming gene (*v-myb*) of avian myeloblastosis virus. Five of the six (not the same exception) contained an abnormally large *myb* RNA transcript.

Mushinski *et al.* deduce from these clues the seemingly unlikely conclusion that transient viral integration caused the rearrangements of *c-myb*, using the exception, mentioned above, as the key evidence. The exceptional plasmacytoid lymphosarcoma contains integrated and transcribed Abelson viral genes in addition to abnormally large transcripts from a rearranged *c-myb*. That, say Mushinski *et al.*, is evidence that the virus was at the scene of the crime in one case, and suggests it was in all, for long enough to hit *c-myb* and run. Supportive evidence of elimination of in-

tegrated Abelson murine leukaemia virus genes from transformed lymphoid cell lines without loss of the transformed phenotype is cited.

As controls, Mushinski *et al.* have studied two other types of lymphoid neoplasm induced by Abelson murine leukaemia virus. One is a plasmacytoma, produced like plasmacytoid lymphosarcomas but more frequently, when mice are pretreated with the immunosuppressive pristane before viral infection. The other control type is the lymphosarcoma induced by viral infection, alone. All examples of both control types had integrated viral DNA and neither rearranged *c-myb* DNA nor abnormally large *myb* RNA.

Whether or not the *c-myb* rearrangements in plasmacytoid lymphosarcomas are caused by transient viral integration, they bear some resemblance to the rearrangements of *c-myc* in murine plasmacytomas and in some human B-cell lymphomas. Indeed, Mushinski *et al.* checked for *c-myc* rearrangements in their plasmacytoid lymphosarcomas and showed them to be absent. Much of the latest evidence concerning *c-myc* rearrangements and abnormal transcripts in such cells was presented at a meeting reported in these columns (*Nature* **302**, 474; 1983). A part of the evidence is now published on page 401 of this issue of *Nature*.

It remains to be seen whether abnormal *c-myc* or *c-myb* transcripts are causally related to the tumours in which they are found, and if so how. □

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Planetary science

When is a meteorite not a meteorite?

from I.P. Wright and C.T. Pillinger

It now seems certain that long before the inception of the Apollo and Viking missions, samples of our nearest neighbours, the Moon and Mars, were already on Earth awaiting discovery. The martian origin for SNC meteorites (named after the 'type' specimens Shergotty, Nahkla and Chassigny and rather unfortunately referred to by the acronym pronounced 'snick' by many in the field) remains somewhat controversial, but for a 32 g meteorite from Antarctica (ALHA 81005), identified during preliminary examinations as an anorthositic breccia, the overwhelming consensus is that it has a lunar origin. Even more surprising than this conclusion, however, is the fact that so many scientists, many of whom met recently in Texas* to discuss the question, have managed to reach agreement on an aspect of extra-terrestrial research in such a short space of time. (The meteorite classification number ALHA 81005 implies that it was collected in 1981; it was only made available for study at the end of 1982.)

The evidence that ALHA 81005 has a lunar origin is manifold. U. Marvin (Harvard University) revealed that members of the field collection party themselves recognized the meteorite as being unusual. At the conference D. Bogard and P. Johnson (NASA, Houston) reported that the trapped noble gases in the meteorite were 'obviously' implanted solar-wind species. The inference is that the matrix was resident at the lunar surface before breccia formation. High concentrations of radiogenic ^{40}Ar and old cosmic-ray exposure ages are taken as evidence that the sample is lunar rather than asteroidal in origin. Further isotopic constraints were provided by T. Mayeda and R. Clayton (University of Chicago) who demonstrated that the $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ data are quite consistent with a lunar origin. Unfortunately no radiometric dating techniques have yet been applied to the sample.

Many authors have made detailed petrographical observations which together with the major- and trace-element data for ALHA 81005 substantiate a lunar origin for the meteorite. The meteorite sample is broadly similar to Apollo 16 lunar breccias, being not particularly rich in titanium, potassium, rare earth elements or phosphorus (P. Warren, G. Taylor and K. Keil, University of New Mexico, Albuquerque; R. Verkonter, J. Dennison and M. Lipschutz, Purdue University, West Lafayette). However on the basis of subtle

differences in some chemical parameters the consensus was that ALHA 81005 originated from a previously unsampled part of the Moon, perhaps the far side.

From the dynamical point of view it is entirely possible for lunar ejecta to reach the Earth. H. Melosh (University of Arizona) demonstrated that unshocked or slightly shocked material can be ejected at velocities which exceed that necessary to escape from the Moon's gravitational field (2.4 km s^{-1}). This is consistent with observations that ALHA 81005 has not been shocked to any greater or lesser extent than some of the samples which were returned to the Earth by the manned Apollo missions. If it could be demonstrated that ALHA 81005 did not originate from the Moon, this would have profound implications, because it would mean there must be another parent body similar in many respects to the Moon. However this is not thought likely.

By contrast with meteorites from the Moon, the SNC meteorites offer the chance to study rocks from a parent body which has never been visited by man. Direct supporting evidence for a martian origin is available from two observations. First, B. Clark (Denver, Colorado) has demonstrated the similarity in chemical composition between martian fines (as measured by the Viking landers) and Shergotty. Second, D. Bogard and P. Johnson (NASA, Houston) have shown that the noble gases trapped in the glass and feldspar fraction of EETA 79001 (an Antarctic meteorite collected at Elephant Moraine and classified as a shergottite) have relative abundances of Ne, Ar, Kr and Xe similar to the martian atmosphere; also some of the noble gas isotope ratios are consistent with this source. However similar data for Chassigny obtained by U. Ott, F. Begemann and P. Lohr (Max-Planck-Institut, Mainz) show a noble gas distribution pattern which is distinctly different from that observed in the martian atmosphere, so in terms of SNC meteorites, perhaps EETA 79001 is the exception to the rule.

Following the lead of the noble gas investigations, results from two independent studies of light-element isotope ratios were presented at the conference. A. Fallick and colleagues (University of Cambridge) have measured $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and δD in the three type meteorites and have found them to be indistinguishable from typical terrestrial values. These data imply that the processes in operation on the parent body were very similar to those taking place on Earth and therefore suggest a planetary origin for the SNC meteorites. In contrast,

the other study by R. Becker, U. Frick and R. Pepin (University of Minnesota) uncovered small amounts of isotopically anomalous nitrogen from the feldspar and glass fraction of EETA 79001. Using the data from the Cambridge investigation, Becker *et al.* have been able to contrive a $\delta^{15}\text{N}$ value of about +600 per mill for the shocked fraction which is consistent with isotopic measurements of the martian atmosphere made by the Viking Landers. Thus SNC meteorites preserve a record in the light-element isotopic compositions of both the lithospheric and atmospheric conditions.

Several indirect lines of evidence that the SNC meteorites had an origin on Mars are of course well established. For example, they have young crystallization ages of about 1.3 Gyr, which requires igneous activity on a large planetary object (not an asteroid) and oxygen isotopic compositions which show that they were formed on a different parent body from the Earth, Moon or the eucrites. The original objection to a martian origin was the belief that it was dynamically impossible to eject rocks from the surface of Mars (which has an escape velocity of 5 km s^{-1}). Ironically it is the discovery of a lunar meteorite that in part undermines previous reservations as it demonstrates that planetary surface ejection events do not require special conditions such as the generation of vapour clouds from indigenous permafrost layers. Indeed A. Singer (SUNY) has shown that even the impact-generated gas cloud mechanism is not sufficient to explain the martian origin for Shergotty. However normal impacts into a dense volatile-rich surface (J. O'Keefe and T. Ahrens, Caltech) or impacts at an oblique angle (L. Nyquist, NASA, Houston) both seem to be capable of explaining the Mars origin for the SNC meteorites.

Recently the SNC meteorite group has gained one member, ALHA 77005, previously classified as a unique achondrite but now identified as a shergottite (J. Smith and I. Steele, University of Chicago) and lost another, Brachina, originally thought to be a chassignite but now considered to be a new type of meteorite (G. Crozaz and P. Pellas, St Louis/Paris; and a consortium of workers based in New York/Mainz). Brachina has been shown to have a different oxygen isotopic composition from all the other SNC meteorites and it also has an early crystallization age (4.5 Gyr) demonstrating that it formed early in the history of the Solar System. There were also fears from some investigators that Chassigny itself may not be an authentic SNC meteorite and so perhaps we should not get into the habit of using the 'snick' colloquialism when referring to this fascinating group of extraterrestrial objects. □

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Kerguelen hotspot source for Rajmahal Traps and Ninetyeast Ridge?

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The hypothesis that hotspots are the sources of many continental flood basalts is evaluated geochemically for the proposed Rajmahal Traps–Ninetyeast Ridge–Kerguelen hotspot system. It appears that the Kerguelen hotspot did not directly feed Rajmahal magmas, although it may have provided a source of heat for Rajmahal activity.

It is still not established where continental flood basalts are formed. Many continental flood basalt provinces lie on or near proposed hotspot tracks and most lavas appear to date from the time the hotspot was in the vicinity (see, for example, ref. 1). This observation is based on combined geochronological, palaeomagnetic, and topographic–bathymetric evidence used in reconstructions of plate motions. The geochemical consequences of such a connection, which could be profound, have not been investigated in any systematic way. This article evaluates geochemical data pertinent to this relationship from a single continental flood basalt province, the present-day (oceanic) expression of its presumed hotspot, and from intervening oceanic locations along the trace of that hotspot.

Specifically, several authors have marshalled evidence suggesting that the Rajmahal Traps of eastern India, the great aseismic Ninetyeast Ridge in the eastern Indian Ocean, and the Kerguelen hotspot located around 50°S (Fig. 1) together form a classic hotspot trace system^{2–5}. With minor variations, the postulated sequence of events in the history of this system is as follows:

- (1) Eruption of the Rajmahal basalts around 110–100 Myr ago^{6,7} as India passed over the hotspot.
- (2) Formation of the >4,500 km long Ninetyeast Ridge from at least 80 Myr to ~38 Myr as the Indian Plate continued northwards, followed by a ridge jump in the Indian Ocean spreading axis placing the hotspot under the Antarctic Plate.
- (3) Construction of the northern end of the Kerguelen Plateau from at least 27 to 5 Myr, based on ages of igneous rocks from the Kerguelen Islands^{8,9}.

An alternative hypothesis for the Rajmahal Traps envisions them as the initial phase of a single long-lived, westward-migrating volcanic disturbance which culminated in the eruption of the Deccan basalts (Fig. 1) around 60–65 Myr ago¹⁰. Marked lithological similarities between the two provinces, the presence of numerous dykes in the intervening region, and lack of contrary evidence have maintained the popularity of this model among Indian geologists.

Further development of either hypothesis would clearly benefit from a geochemical and isotopic comparison of all four areas. In the first case, similarities between the Rajmahal Traps, Ninetyeast Ridge, and the Kerguelen Islands would be expected; while if the second model pertained, the Rajmahal basalts might display strong affinities with the Deccan Traps.

Lavas and intrusives from Kerguelen have recently been analysed for Nd and Sr isotopes by Dosso and Murthy¹¹ and

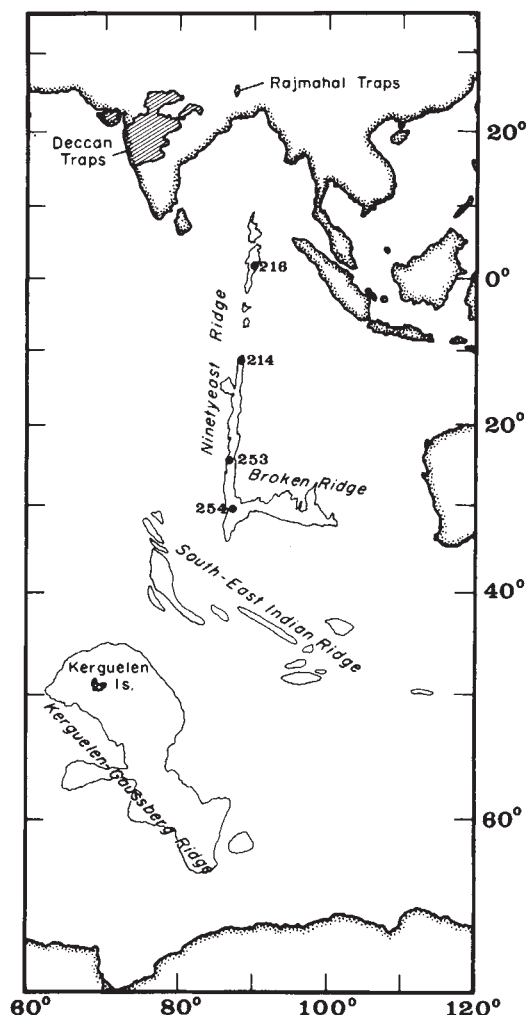


Fig. 1 Map of the eastern Indian Ocean and bordering land masses. The Ninetyeast Ridge, Kerguelen Plateau, and Southeast Indian Ocean Ridge are delineated by the 3-km depth contour. Locations of DSDP sites are also shown.

White and Hofmann¹². They are characterized by very heterogeneous, though very distinctive isotopic compositions ranging from $\epsilon_{\text{JUV}} \sim +6$ to -6 , $^{87}\text{Sr}/^{86}\text{Sr} \sim 0.7039\text{--}0.7059$, well beyond the range of mid-oceanic ridge basalts (MORB) ($\epsilon_{\text{JUV}} \geq +10$) and of most other oceanic islands (average $\epsilon_{\text{JUV}} \sim +6$). The mantle source for the western Deccan Traps appears strikingly different. We have recently analysed some Deccan flows both isotopically and chemically (ref. 13, and unpublished data). Those least contaminated by continental crust point to a relatively homogeneous source, with $\epsilon_{\text{JUV}} \geq +8$ and enriched in radiogenic Sr, plotting slightly to the right of the Nd–Sr mantle array.

Sr isotopes have been measured from locations along the Ninetyeast Ridge by Whitford and Duncan¹⁴ and Reddy *et al.*¹⁵; the former concluded that a strong similarity indeed exists with Kerguelen. Unfortunately, essentially all lavas dredged or drilled on the Ninetyeast Ridge are altered to varying degrees by exposure to seawater (very little fresh glass exists in these dominantly subaerially-erupted crystalline flows); therefore arguments based on high $^{87}\text{Sr}/^{86}\text{Sr}$ or alteration-prone trace element ratios¹⁵ obtained from whole rocks are weakened considerably by the likelihood of contamination. Nd isotopic compositions are relatively immune to such effects but have not been determined previously. Fairly abundant trace element data exist for the Ninetyeast Ridge^{16–21}; however, there is a dearth of comparable data for the Kerguelen Islands, particularly for elements resistant to seawater alteration (such as, Zr, Nb, Y, rare-earth elements). Thus, studies of Ninetyeast Ridge trace elements were unable to address the Kerguelen hotspot hypothesis directly.

The Rajmahal Traps have remained more or less a geochemical frontier; essentially no previous trace element or Nd or Sr isotopic work exists. Accordingly, in early 1982 we collected samples from the Rajmahal Traps and obtained core samples from various eruptive units of DSDP sites along the Ninetyeast Ridge for detailed chemical and Nd and Sr isotopic analysis. Our analytical techniques were identical to those reported in ref. 13.

Rajmahal Traps

Lesser known and much less extensive than the vast Deccan province of western and central India, the Rajmahal Traps have been substantially reduced in size by downfaulting and burial to the east, and by erosion^{6,22}. Presently they consist of between 10 (refs 23, 24) and 29 (C. Ramaswamy, personal communication) gently eastward-dipping flows totalling at least 600 m in thickness. Rock types vary from tholeiitic basalts to basaltic andesites and include at least one fairly widespread flow of silicic pitchstone²⁵. A detailed petrographic summary has been given by Deshmukh²⁵. Our analyses of the freshest available samples (Table 1) indicate a relatively wide range of MgO/FeO^* (0.44–0.91), $\text{CaO}/\text{Al}_2\text{O}_3$ (0.40–0.76), SiO_2 (50.1–58.1%), and in particular, of the highly LIL (large ion lithophilic) elements K (910–7,470 p.p.m.), Rb (0.53–40.5 p.p.m.), and Ba (61–268 p.p.m.). Variation of Ni with Ti and MgO/FeO^* points to substantial differences in degrees and possible pathways of differentiation. Nevertheless, the total range of fractionation is smaller and along significantly different lines of descent than for the Deccan basalts at Mahabaleshwar, some of which attain extreme values of FeO^* and TiO_2 enrichment (as much as 16.34% and 4.29%, respectively)¹³. The Rajmahal lavas also appear to be richer in SiO_2 than the average Deccan basalt, and in general LIL trace element ratios such as Ba/Zr and K/Rb do not approach the highly depleted, MORB-like values of many of the Mahabaleshwar flows.

Initial isotopic ratios for the Rajmahal samples vary from $\epsilon_{\text{JUV}}(T) = +4.5$ to -3.4 , $(^{87}\text{Sr}/^{86}\text{Sr})_i = 0.70412$ to 0.70710 , and form a well-defined, nearly linear trend oriented away from the positive ϵ_{JUV} region of the Nd–Sr mantle array towards negative ϵ_{JUV} , high $^{87}\text{Sr}/^{86}\text{Sr}$ values (Fig. 2). Plots of two LIL trace element ratios typically display good, in some cases excellent, correlations. In general, there are also good correlations

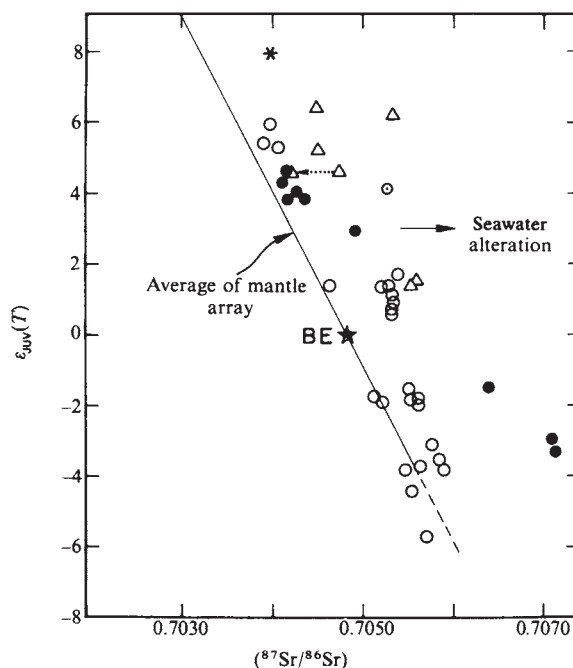


Fig. 2 Initial Nd versus Sr isotopic ratios of Rajmahal Traps and Ninetyeast Ridge samples. Also plotted are the average line of the trend defined by mantle-derived rocks, the presumed bulk Earth value (BE), and the position of the least contaminated Deccan samples from Mahabaleshwar (★). ●, Main-sequence Rajmahal lavas; ○, RM82-5; △, Ninetyeast Ridge samples; ○, Kerguelen igneous rocks. ϵ values for Kerguelen, taken from refs 11 and 12, are present-day values ($\epsilon_{\text{JUV}}(0)$), but all samples are younger than ~27 Myr; thus age corrections are negligible. Sr isotopic ratios from ref. 11 are initial values, while those from ref. 12 are not. Again, the differences are minimal. For the Ninetyeast Ridge, initial $\epsilon_{\text{JUV}}(T)$ and approximate 'initial' $(^{87}\text{Sr}/^{86}\text{Sr})_i$ values are shown. Conversion of Nd isotopic compositions reported in refs 11 and 12 to ϵ_{JUV} notation is accompanied by a probable uncertainty of $\pm 0.5 \epsilon$ units owing to uncertainties in interlaboratory calibrations. Dotted arrow represents effect of acid leaching in an ultrasonic bath for ~2 h on sample 254-36-3.

between isotopic ratios and LIL trace element ratios involving Ba, Rb, or K—which exhibit large variations in concentration—and an element varying by a factor of about two or less, such as Zr, Nd, Y, or Nb (Fig. 3). Poorer correlations are observed with ratios of two of the latter group of elements, for example, Sm/Nd, Nb/Zr, or Zr/Y. Not surprisingly, isotopic ratios correlate fairly well with Ba, Rb, and K concentrations (despite effects of differential weathering, late stage fluid migration, and so on), while only poorly with Nd, Sm, Zr, Nb, Y, P, and Ti.

The most straightforward interpretation of these results, given that the Rajmahal Traps rest on a thick Precambrian crust (see, for example, ref. 22), is that they reflect contamination of magmas from a long-term LIL-depleted mantle source by LIL-enriched continental crust. Fundamentally similar relationships have been observed for the Deccan Traps at Mahabaleshwar¹³, the Columbia River basalts²⁶, and the North Atlantic Tertiary Province²⁷. The tight correlation of the points in Fig. 2 indicates that the mantle source region was relatively homogeneous and that a discrete crustal endmember was responsible for most of the contamination. The implication of the trace element data is that the crustal contaminant was greatly enriched compared with the uncontaminated magma in the first group of elements (Ba, K, and Rb), probably by at least one or two orders of magnitude, while concentrations of the second group of elements were much more alike in the two

Table 1 Chemical and isotopic analyses of Rajmahal Traps and Ninetyeast Ridge volcanics

Sample no.	RM82-3	RM82-4	RM82-5	RM82-7	RM82-8	RM82-9	RM82-10	RM82-11	RM82-12	RM82-13	RM82-14	RM82-16	RM82-17
SiO ₂ (%)	52.08	52.42	50.12	51.34	51.73	53.25	58.11	53.09	54.29	51.96	53.55	51.00	50.94
TiO ₂	1.32	1.64	1.36	1.49	1.72	1.84	0.99	1.65	1.48	1.76	1.69	2.13	2.02
Al ₂ O ₃	16.22	15.19	16.23	15.97	15.04	14.35	15.88	15.00	16.12	14.14	14.25	14.25	15.40
FeO*	9.91	10.74	10.08	10.30	10.80	10.63	7.69	10.24	9.20	11.72	11.14	12.51	11.68
MgO	6.53	5.83	6.96	6.34	6.28	6.02	7.02	6.04	5.18	6.20	5.77	5.85	5.24
CaO	11.39	10.56	11.22	11.36	10.78	10.31	6.41	9.49	9.24	10.77	10.01	10.91	10.74
Na ₂ O	2.38	2.63	2.13	2.35	2.52	2.41	3.24	2.64	2.93	2.57	2.55	2.40	2.66
P ₂ O ₅	0.07	0.14	0.14	0.15	0.20	0.23	0.14	0.24	0.24	0.20	0.20	0.27	0.27
K (p.p.m.)	1,660	1,660	913	2,407	1,245	1,826	1,992	7,470	6,640	1,494	2,988	1,079	1,245
Rb	7	3.64†	0.53†	4.84†	1.71†	3	4	18.4†	40.5†	5	6	2.93†	4
Ba	84	130	96	83	76	72	68	251	268	91	102	68	67
Nb	3	6	4	4	6	7	5	12	12	7	7	7	8
Zr	83	114	76	84	110	122	106	159	155	123	122	126	118
Y	28	35	30	29	37	40	34	39	36	40	42	41	41
Sr	227	239.3†	231.4†	230.4	228.7†	282	165	324.2†	323.1†	225	204	226.3†	245
Ni	88	78	86	82	67	54	125	38	34	65	63	75	67
V	268	312	160	277	288	290	132	230	240	317	330	330	322
Nd†		12.65	8.63	9.42	12.12			20.11	20.87			14.52	
Sm†		3.98	2.82	3.04	3.83			5.07	5.15			4.33	
$\epsilon_{\text{JUV}}(T)^\ddagger$		+2.9±0.2	+4.1±0.4	+3.9±0.4	+4.5±0.2			-3.4±0.4	-3.0±0.4			+4.3±0.2	
(⁸⁷ Sr/ ⁸⁶ Sr) _i ‡		0.70494	0.70525	0.70427	0.70416			0.70710	0.70708			0.70412	
		±0.00002	±0.00003	±0.00002	±0.00001			±0.00003	±0.00002			±0.00003	

Sample no.	RM82-19	R-63	R-43	R-48	R-20	216-37-2 (~118 cm)	216-38-2 (~132 cm)	214-50-1 (~130 cm)	214-53-1 (~95 cm)	214-54-3 (~133 cm)	253-58-CC	254-36-3 (~115 cm)	Estimated errors§
SiO (%)	51.73	50.86	52.05	52.38	53.44	50.27	51.07	58.36	49.01	49.69	46.02	47.19	0.20
TiO ₂	1.97	2.31	1.93	1.77	1.24	2.85	2.70	1.39	2.08	2.16	0.75	2.53	0.04
Al ₂ O ₃	15.34	14.79	15.43	15.71	15.87	14.71	13.82	16.22	15.21	14.88	16.99	15.11	0.15
FeO*	10.93	11.74	10.83	10.23	8.10	11.72	12.71	8.70	14.91	13.14	10.03	13.96	0.16
MgO	5.56	6.23	5.47	5.40	6.95	5.63	5.69	2.33	7.11	6.06	12.99	7.68	0.08
CaO	10.66	11.00	10.61	9.91	10.16	9.83	9.40	5.91	8.34	10.47	10.69	10.02	0.08
Na ₂ O	2.55	2.59	3.12	2.81	2.62	2.84	2.58	4.10	1.93	2.59	1.05	2.52	0.06
P ₂ O ₅	0.21	0.19	0.26	0.20	0.18	0.32	0.26	0.84	0.17	0.21	0.03	0.29	0.03
K (p.p.m.)	1,826	1,660	1,909	3,403	4,399	3,520	4,240	11,280	2,400	1,280	1,360	1,120	100
Rb	2	4	6.68†	6.19†	19.8†	3.47†	9.26†	39.3†	5.66†	3	2.74†	0.69†	2
Ba	89	61	110	74	180	89	102	268	7	11	10	44	5
Nb	7	7	7	6.5	8	26	24	39	15	15	7	19	2
Zr	125	115	113	104	115	178	166	349	142	156	34	159	7
Y	39	40	35	33	30	34	40	70	40	39	33	49	3
Sr	221	287	241.3†	215.5†	330.3†	192.6†	177.1†	282.9†	111.4†	181	75.55†	172.4†	4
Ni	79	58	52	61	39	43	42	3	62	48	376	150	4
V	320	330	304	313	220	486	466	42	375	442	288	367	6
Nd†			16.11	14.17	16.26	20.22	19.38	46.48	14.91		4.30	17.23	
Sm†			4.86	4.21	4.16	5.31	5.08	11.09	4.35		1.72	4.94	
$\epsilon_{\text{JUV}}(T)^\ddagger$			+3.8±0.4	+3.8±0.3	-1.5±0.5	+1.5±0.2	+1.4±0.4	+5.2±0.2	+6.4±0.3		+6.2±0.6	+4.6±0.5	
(⁸⁷ Sr/ ⁸⁶ Sr) _i ‡			0.70433	0.70415	0.70638	0.70556	0.70552	0.70450	0.70466		0.70533	0.70473	
			±0.00003	±0.00002	±0.00003	±0.00003	±0.00002	±0.00003	±0.00003		±0.00003	±0.00003	

† Measured by isotope dilution: Sr determined by X-ray fluorescence (XRF) generally agrees within 2%.

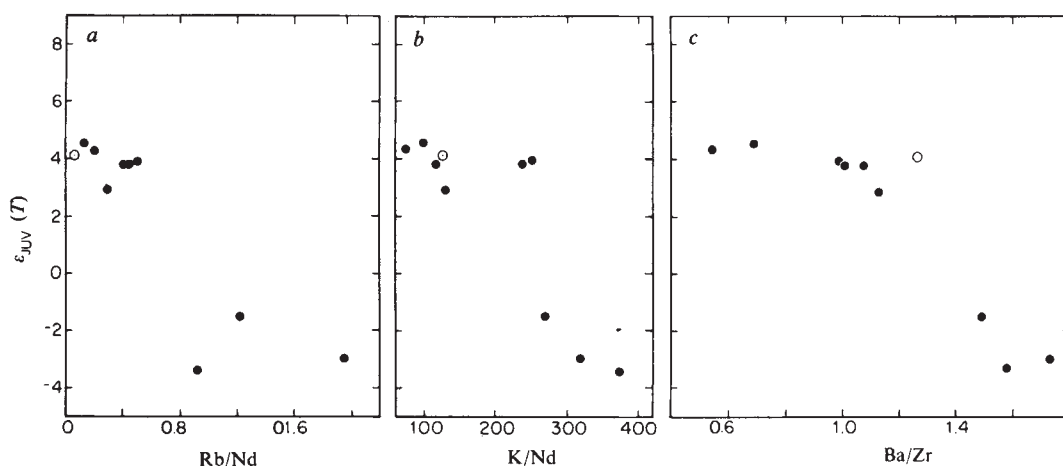
Rb is within 2 p.p.m. (average) when measured by XRF and isotope dilution.

Sm and Nd estimated precision is better than 0.2%.

‡ An age correction of 100 Myr was applied to Rajmahal samples, 70 Myr to Site 216, 60 Myr to Site 214, 50 Myr to Site 253, and 40 Myr to Site 254 samples.

§ σ_m values are listed for major elements. For trace elements maximum deviations in p.p.m. from XRF analyses of duplicate pellets are given.Isotopic fractionation corrections: $^{86}\text{Sr}/^{88}\text{Sr} = 0.1194$, $^{148}\text{NdO}/^{144}\text{NdO} = 0.242436$. A value of $^{87}\text{Sr}/^{86}\text{Sr} = 0.71025$ is measured for NBS 987 Sr, with a total range of 0.000026. Nd values are given relative to $^{143}\text{Nd}/^{144}\text{Nd} = 0.511859$ for the La Jolla Nd standard, such that $^{143}\text{Nd}/^{144}\text{Nd} = 0.512566$ for present-day Juvinas which is defined as $\epsilon_{\text{JUV}}(0) = 0$.

$$\epsilon_{\text{JUV}}(T) = \left[\frac{^{143}\text{Nd}/^{144}\text{Nd}_{\text{sample at time } T}}{^{143}\text{Nd}/^{144}\text{Nd}_{\text{JUVINAS at time } T}} - 1 \right] \times 10^4$$

Fig. 3 a, $\epsilon_{\text{JUV}}(T)$ versus Rb/Nd; b, K/Nd; c, Ba/Zr for Rajmahal lavas. Symbols are as in Fig. 2. An ideal mixing array in a and b would be linear, because the denominator for both x and y axes is Nd. For these and similar plots extrapolation along the x-axis to either 0 or likely minimum mantle ratios indicates a mantle source with $\epsilon_{\text{JUV}}(T) \approx +6$.

endmembers. Very similar situations have been documented for the North Atlantic Tertiary Province²⁸ and the western Deccan²⁹. Whether the crustal endmember was assimilated in bulk or represented the early melting portions of wallrock or wallrock xenoliths cannot be ascertained at present. In any case, mixing trends arising in the second group of elements could easily have been overprinted by effects of fractionation, slightly different amounts of partial melting, and/or minor inhomogeneities in the source (or crustal contaminant); their usefulness is thus for indicating processes other than contamination.

One sample in Fig. 2, RM82-5, lies well to the right of the others, implying (in the above framework) contamination by a different crustal endmember. Assuming that its mantle source was identical to that of the other samples, then for any reasonable crustal contaminant a common mantle endmember must have possessed an ϵ_{JUV} of at least about +6. In other words, the intersection of almost any hypothetical mixing curve passing through RM82-5 with the extension of the trend formed by the remaining lavas yields a common mantle source for all these basalts with $\epsilon_{\text{JUV}} \geq +6$. Consideration of combined trace element and isotopic data such as those in Fig. 3, on the other hand, allows us to place an approximate upper limit on the ϵ_{JUV} value of the mantle endmember somewhere around +6. Because isotopic evidence alone suggests a probable minimum near this value, it therefore appears relatively well constrained. Furthermore, the extrapolated $(^{87}\text{Sr}/^{86}\text{Sr})_i$ of the mantle source region, or even the measured values of samples with $\epsilon_{\text{JUV}}(T) > +3.5$, put it well within the Nd-Sr mantle array. The Rajmahal source was therefore quite distinct from that of the western Deccan¹³. This result, in agreement with recent evidence from K-Ar dating in both provinces⁶, tends to discredit the view relating both to a single westward-moving centre of volcanism.

Finally, there appears to be no simple relationship between degree of fractionation and amount of contamination. For example, samples R-20, RM82-11, and RM82-12 are the most contaminated based on their isotopic compositions and low Ni contents. All are essentially aphyric. However, R-20 appears substantially less differentiated than the other two judging from its MgO/FeO^* (0.86 compared with 0.59 and 0.56, respectively) and its generally lower LIL element values (Table 1). Combined fractionation and assimilation might be expected to result in the most contaminated flows of a sequence being also the most fractionated^{30,31}. Such may indeed be the case for the Columbia River basalts²⁶; yet the most contaminated Deccan flows at Mahabaleshwar actually appear to be the least fractionated¹³. O'Hara's³² suggestion that primitive unfractionated magmas may be $\sim 250^\circ$ hotter than more differentiated ones, along with recent analytical models³³ indicating the highest degrees of contamination in flows erupted early in the lifetime of a magma chamber, may be pertinent in this respect. Consideration of recent experiments demonstrating the likelihood of early selective contamination³⁴, as well as possibilities of periodically replenished magma systems or multiple magma chambers emphasizes the probable complexity of real-world contamination processes.

Ninetyeast Ridge

The Ninetyeast Ridge constitutes the longest aseismic ridge in the world; its unique morphological-tectonic features have been reviewed by Curray *et al.*⁴. Four DSDP holes along the ridge crest penetrated to volcanic basement, the flows of which appear to have been erupted either subaerially or in very shallow water. Detailed petrographic and geochemical descriptions of these rocks are given in the Legs 22 and 26 *Initial Reports of the Deep Sea Drilling Project*¹⁶⁻¹⁹, and are summarized by Frey *et al.*²⁰ and Ludden *et al.*²¹.

The results of our work are listed in Table 1 and depicted graphically in Figs 2 and 4. Also plotted in Fig. 2 are previously determined isotopic compositions for igneous rocks from Kerguelen^{11,12}. $\epsilon_{\text{JUV}}(T)$ values from the Ninetyeast Ridge vary widely from +1.4 at Site 216 to +6.4 at Site 214 and overlap

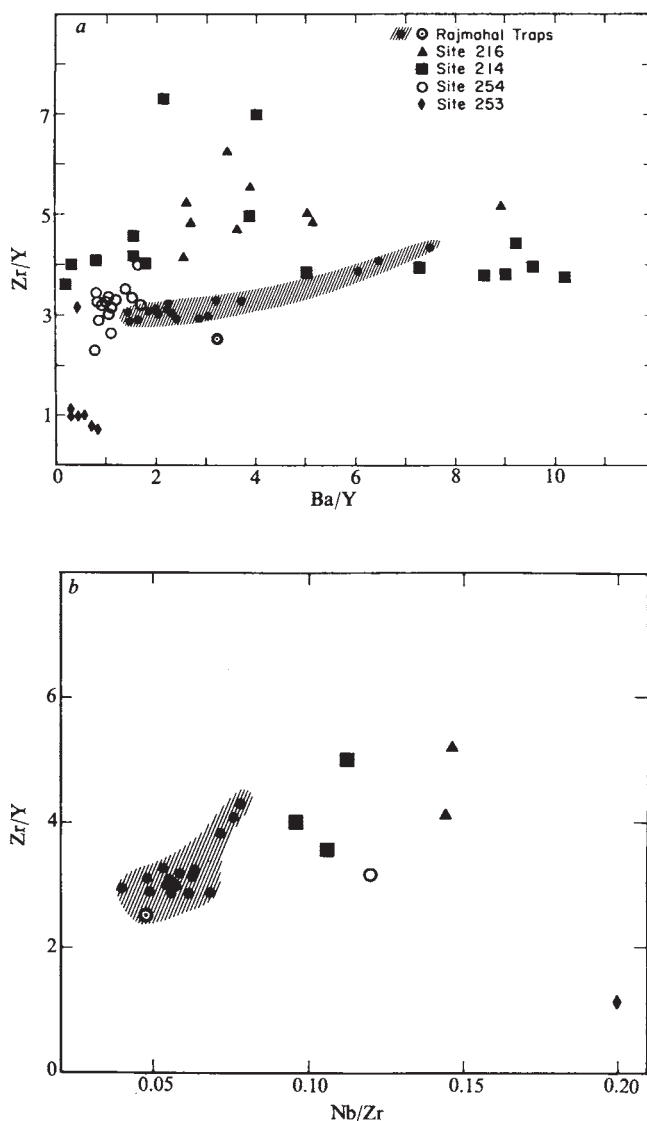


Fig. 4 a, Zr/Y versus Ba/Y for the Rajmahal and Ninetyeast Ridge volcanics, including previously published values for the Ridge¹⁶⁻²⁰. Site 214 samples with high Ba/Y are oceanic andesites. Note that the distribution of Rajmahal points approximates a mixing array between a mantle endmember having Zr/Y around ~ 2.7 and a crustal contaminant in the upper right of the diagram. Some of the scatter in Ba/Y values for the Ninetyeast Ridge probably results from the influence of seawater alteration on Ba. b, Nb/Zr versus Zr/Y for the Rajmahal Traps and Ninetyeast Ridge samples from this study. To our knowledge, no previous Nb measurements for the Ninetyeast Ridge exist.

with those from Kerguelen, which display an even wider range. For each of our samples, an 'initial' $(^{87}\text{Sr}/^{86}\text{Sr})_i$ was calculated using measured Rb and Sr concentrations with the realization that seawater interaction had probably changed both concentrations and Sr isotopic compositions to some extent. These values lie between 0.70450 and 0.70556. Figure 2 shows that all Ninetyeast Ridge points are displaced to the right of the mantle array—and the field of Kerguelen rocks—towards the $^{87}\text{Sr}/^{86}\text{Sr}$ of seawater (0.709 today). Alteration effects are clearly visible in thin sections, and X-ray diffraction patterns revealed the presence of poorly crystallized smectite or chlorite in all samples (and in some cases, minor calcite). A powdered aliquant of one sample, 254-36-3, was leached in $\sim 2\text{M HCl}$ for $\sim 1/2$ h in an ultrasonic bath. A significant reduction in $(^{87}\text{Sr}/^{86}\text{Sr})_o$ was observed, from 0.70473 to 0.70449, and no clay peaks could

be discerned on an X-ray diffraction trace of the residue. Further leaching (2 h) resulted in an even greater reduction to 0.70421. Nd isotopic values remained unchanged. All of this strongly suggests that the measured $^{87}\text{Sr}/^{86}\text{Sr}$ values have been increased by interaction with seawater and do not represent initial magmatic values.

Hotspot hypothesis

In general the isotopic data appear consistent with a Kerguelen hotspot origin for the Ninetyeast Ridge. Certainly the very low $\epsilon_{\text{JUV}}(T)$ and high ($^{87}\text{Sr}/^{86}\text{Sr}$)_i (even after allowance for seawater alteration) of Site 216 appear to be outside the range of other Indian Ocean islands^{14,35}; in fact, few other oceanic islands in the world approach such extreme isotopic compositions (see, for example, ref. 12). The Site 216 basalts are also the oldest of the four Ridge sites (≥ 70 Myr). Thus, the expression of a LIL-enriched mantle reservoir apparent during the past 27 Myr in the Kerguelen Islands evidently has persisted over a much greater interval of time. A number of points from Kerguelen fall between $\epsilon_{\text{JUV}}(0) \sim -1$ and -6 . Significantly, most of these appear to be from strongly alkalic or acidic rocks,¹¹ while the samples with higher $\epsilon_{\text{JUV}}(0)$ values up to $\sim +6$ measured by White and Hofmann¹² are mainly tholeiitic or olivine basalts⁸, as are the Ninetyeast Ridge volcanics. In any case, the overlap in ϵ_{JUV} between the Kerguelen and Ninetyeast Ridge fields is substantial, and considerable isotopic heterogeneity appears to be a keynote feature of both Kerguelen and the Ridge. Heterogeneity at Kerguelen has been ascribed to heterogeneity in the mantle source^{9,11,12}, there being no evidence for continental basement under Kerguelen^{9,11}; nor is the Ninetyeast Ridge underlain by continental crust (see, for example, ref. 5). Therefore, although the variation in isotopic values may be superficially similar to that of the Rajmahal Traps (and other continental flood basalts), the mechanism of continental crustal contamination which best explains the continental basalt data cannot account for the results from these two oceanic provinces.

Not surprisingly, alteration-resistant trace element ratios from the Ninetyeast Ridge exhibit a comparable range of heterogeneity. As illustrated in Fig. 4, Zr/Y and Nb/Zr scatter widely, not only from hole to hole, but also among the different units of a single site. Isotopic data corroborate this tendency: a difference of 1.2 ϵ_{JUV} units is observed between two closely spaced flows, 214-50-1 and 214-53-1. The former is an 'oceanic andesite' previously thought to be simply a more evolved product of the same source that gave rise to the earlier

ferrobasalts^{15,20,21}. Interestingly, the light REE-depleted picritic sample from site 253 could not have issued from a typical MORB-type mantle, as has been argued previously^{20,21}, being quite distinct in its Nb/Zr, Zr/Y, and isotopic ratios.

The Rajmahal Traps present a strikingly different aspect than the Ninetyeast Ridge. For a given $\epsilon_{\text{JUV}}(T)$, Sm/Nd ratios for the Rajmahal basalts are consistently and significantly higher than values for either the Ninetyeast Ridge or Kerguelen (using data from ref. 11, and W. White, personal communication). In Fig. 4b the Rajmahal basalts fall into a restricted field completely separated from any of our Ninetyeast Ridge samples, while in Fig. 4a they are distinguished by the very coherence of their distribution, which approximates a mixing array. In agreement with the isotopic data, Fig. 4 indicates that the Rajmahal mantle source region was quite homogeneous, in contrast to that of the Ninetyeast Ridge or Kerguelen. The weight of evidence presented here therefore seems to rule out any simple connection between the Ninetyeast Ridge and the Rajmahal basalts.

While a Kerguelen hotspot origin thus seems likely for the Ninetyeast Ridge, it appears improbable that the hotspot furnished magmas for Rajmahal lavas. The possibility remains, however, that the hotspot may have operated as a heat source for Rajmahal activity without directly providing material; this may in fact be a more usual relation between flood basalts and nearby hotspots. Morgan¹ has argued that the heaviest eruptive activity commonly occurs only when a hotspot is at the trailing edge of a continental flood basalt province, after it has passed dormant beneath the region for many millions of years. If the model discussed here is at all valid, his observation and the present data suggest a gradual heating, weakening, and eventual melting of the subcontinental mantle by a hotspot, rather than hotspot penetration of the continental crust. As a consequence, this would mean that a large portion of the subcontinental mantle beneath the Rajmahal Traps was not only LIL-depleted, but also fell within the Nd-Sr mantle array defined by volcanic rocks from suboceanic mantle sources.

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1. Morgan, W. J. in *The Sea* Vol. 7 (ed. Emiliani, C.) 443-487 (Wiley, New York, 1981).
2. Pierce, J. W. *Geophys. J. R. astr. Soc.* **52**, 277-311 (1978).
3. Duncan, R. A. *J. Volcan. Geotherm. Res.* **4**, 283-305 (1978).
4. Curran, J. R., Emmel, F. J., Moore, D. G. & Raitt, R. W. in *The Ocean Basins and Margins* Vol. 6 (eds Nairn, A. E. M. & Stehli, F. G.) (Plenum, New York, 1983).
5. Subbarao, K. V. & Reddy, V. V. *Tectonophysics* **75**, 69-89 (1981).
6. Agrawal, J. K. & Rama, F. A. *Proc. Ind. Acad. Sci.* **84A**, 157-179 (1976).
7. McDougall, I. & McElhinny, M. W. *Earth planet. Sci. Lett.* **9**, 371-378 (1970).
8. Watkins, N. D., Gunn, B. M., Nougier, J. & Baksi, A. K. *Bull. geol. Soc. Am.* **85**, 201-212 (1974).
9. Dosso, L. et al. *Earth planet. Sci. Lett.* **43**, 46-60 (1979).
10. Pascoe, E. H. *A Manual of the Geology of India and Burma*, Vol. 3, 1399 (Government India Press, 1964).
11. Dosso, L. & Murthy, V. R. *Earth planet. Sci. Lett.* **48**, 268-276 (1980).
12. White, W. M. & Hoffmann, A. W. *Nature* **296**, 821-825 (1982).
13. Mahoney, J. J. et al. *Earth planet. Sci. Lett.* **60**, 47-60 (1982).
14. Whitford, D. J. & Duncan, R. A. *Yb. Carnegie Instn Wash.* **77**, 606-613 (1978).
15. Reddy, V. V., Subbarao, K. V., Reddy, G. R., Matsuda, J. & Hekinian, R. *Mar. Geol.* **26**, 99-117 (1978).
16. Hekinian, R. *Init. Rep. DSDP* **22**, 413-447 (1974).
17. Thompson, G., Bryan, W. B., Frey, F. A. & Sung, C. M. *Init. Rep. DSDP* **22**, 459-468 (1974).

18. Kempe, D. R. C. *Init. Rep. DSDP* **26**, 465-503 (1974).
19. Frey, F. A. & Sung, C. M. *Init. Rep. DSDP* **26**, 567-572 (1974).
20. Frey, F. A., Dickey, J. S., Thompson, G. & Bryan, W. B. in *Indian Ocean Geology and Biostrophigraphy*, 189-257 (eds Hiertzler, J. R. et al.) (American Geophysical Union, Washington DC, 1977).
21. Ludden, J. N., Thompson, G., Bryan, W. B. & Frey, F. A. *J. geophys. Res.* **85**, 4405-4420 (1980).
22. Krishnan, M. S. *Geology of India and Burma* 4th edn (Higginbothams, Madras, 1960).
23. Ball, V. *Mem. geol. Surv. Ind.* **13**, Art. 2, 155-248 (1877).
24. Hobson, G. V. *Rec. geol. Surv. Ind., Gen. Rep.* **62**, 145-146 (1929).
25. Deshmukh, S. S. *22nd int. Geol. Congr. Pt. 7*, 61-84 (1964).
26. Carlson, R. W., Lugmair, G. W. & Macdougall, J. D. *Geochim. cosmochim. Acta* **45**, 2483-2500 (1981).
27. Carter, S. R., Evensen, N. M., Hamilton, P. J. & O'Nions, R. K. *Nature* **281**, 28-30 (1979).
28. Thompson, R. N., Dickinson, A. P., Gibson, I. L. & Morrison, M. A. *Contr. Miner. Petrol.* **79**, 159-168 (1982).
29. Najafi, S. J., Cox, K. G. & Sukheswala, R. N. *Mem. Geol. Soc. Ind.* **3**, 300-315 (1980).
30. O'Hara, M. J. *Phil. Trans. R. Soc. A* **297**, 215-227 (1980).
31. DePaolo, D. J. *Earth planet. Sci. Lett.* **53**, 189-202 (1981).
32. O'Hara, M. J. *Phil. Trans. R. Soc. A* **288**, 627-629 (1978).
33. Furlong, K. P. & Myers, J. B. *EOS* **63**, 458 (1982).
34. Watson, E. B. *Contr. Miner. Petrol.* **80**, 73-87 (1982).
35. Hedge, C. E., Watkins, N. D., Hildreth, R. A. & Doering, W. P. *Earth planet. Sci. Lett.* **21**, 29-34 (1973).

A glial progenitor cell that develops *in vitro* into an astrocyte or an oligodendrocyte depending on culture medium

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We have identified a cell type in 7-day-old rat optic nerve that differentiates into a fibrous astrocyte if cultured in the presence of fetal calf serum and into an oligodendrocyte if cultured in the absence of serum. In certain culture conditions some of these cells acquire a mixed phenotype, displaying properties of both astrocytes and oligodendrocytes. These observations suggest that fibrous astrocytes and oligodendrocytes develop from a common progenitor cell and provide a striking example of developmental plasticity and environmental influence in the differentiation of CNS glial cells.

NEUROEPITHELIAL cells of the neural tube are thought to give rise to the neurones, astrocytes, oligodendrocytes and ependymal cells that form the vertebrate central nervous system (CNS)¹. However, it is not known what determines the choice of differentiation pathway taken by individual neuroepithelial cells nor when these choices are made. Progress in understanding neural cell lineages and the mechanisms and timing of neural cell determination has been severely impeded by the complexity and inaccessibility of the developing vertebrate CNS and by the uncertainties involved in distinguishing one immature cell type from another by morphological criteria. In the experiments reported here, we have used three strategies to help overcome these problems: (1) we have studied the simplest part of the CNS, the optic nerve, which contains glial cells but no neuronal cell bodies; (2) we have used dissociated cell cultures to control the cellular and fluid environment during glial cell development; (3) we have used antibodies to identify and manipulate the developing glial cells. In this way, we have been able to demonstrate for the first time plasticity in the development of glial cells in the vertebrate CNS: we show that a glial progenitor cell in neonatal rat optic nerve can be induced to develop into a fibrous astrocyte or an oligodendrocyte depending on the culture medium.

Two types of astrocyte

Astrocytes contain characteristic intermediate filaments, called glial filaments, which are largely polymers of glial fibrillary acidic protein (GFAP)^{2,3}. Thus, these cells can be readily identified in tissue sections and cultures of CNS by immunohistochemical techniques using anti-GFAP antibodies²⁻⁴. We have previously reported that two different types of GFAP⁺ astrocytes can be distinguished in cultures of developing rat optic nerve on the basis of morphology, growth characteristics and labelling with ligands that bind to polysialogangliosides, such as tetanus toxin and the monoclonal antibody A2B5⁵. These ligands have been reported to bind to the gangliosides GD1b and GT1 and GQ, respectively^{6,7}, and were originally thought to bind only to neurones⁷⁻⁹. Type 1 astrocytes have a fibroblast-like morphology (Fig. 1a, b), proliferate in culture (especially in response to epidermal growth factor (EGF) or growth factors present in an extract of bovine pituitary) and do not bind detectable amounts of tetanus toxin or A2B5 antibody. Type 2 astrocytes have a process-bearing morphology, resembling neurones or oligodendrocytes (Fig. 1c-h), divide infrequently in culture (even in the presence of EGF or bovine pituitary extract) and bind tetanus toxin and A2B5

antibody. Whereas type 1 astrocytes are found in cultures of both CNS white matter and grey matter, type 2 astrocytes are seen in significant numbers only in cultures of white matter and probably correspond to fibrous (fibrillary) astrocytes (R.H.M. and M.C.R., in preparation). We showed that most type 2 astrocytes in cultures of optic nerve develop from A2B5⁺, GFAP⁻ precursor cells, which rapidly acquire GFAP in culture⁵.

Because type 2 astrocytes divide infrequently in optic nerve cultures, they are quickly overgrown by rapidly dividing cells from the meninges that surround the optic nerve. In an attempt to maintain type 2 astrocytes for longer periods *in vitro*, we cultured optic nerve cells in chemically defined, serum-free medium¹⁰. To our surprise, very few type 2 astrocytes developed in these cultures, while many more oligodendrocytes developed than normally did so in serum-containing medium. The oligodendrocytes were identified using antibodies against galactocerebroside (GC), the major glycolipid in myelin, which is thought to be a specific cell-surface marker for oligodendrocytes in cultures of vertebrate CNS^{4,11} (Fig. 1f-h). This observation was the starting point for the experiments reported here.

Effects of serum on glial cell development

Optic nerves were dissected from 7-day S/D rats, dissociated with collagenase and trypsin, and cultured on poly-L-lysine (PLL)-coated glass coverslips, or PLL-coated plastic dishes as previously described^{4,5}, except that the optic nerves were cut into small pieces before enzyme treatment to increase the yield of type 2 astrocytes. Cultures were grown in either Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated fetal calf serum (FCS), or in serum-free DMEM with various additives as described by Bottenstein and Sato¹⁰ (B-S medium). After 3 days in culture, the cells were studied by indirect immunofluorescence using two fluorochromes, either directly on glass coverslips, or after they had been removed from the plastic dishes by trypsin and EDTA as previously described^{4,5}. The cells were incubated in either monoclonal A2B5 antibody⁷ (ascites fluid, 1:50) or monoclonal anti-GC antibody¹² (ascites fluid, 1:50) followed by goat anti-mouse immunoglobulin conjugated to rhodamine (G anti-MIg-Rd, 1:100, Cappel); after washing, they were fixed in 5% glacial acetic acid in ethanol (acid-alcohol) and incubated with rabbit anti-GFAP (1:1,000) followed by goat anti-rabbit immunoglobulin conjugated to fluorescein (G anti-RIg-FI, 1:80, Nordic).

After 3 days in DMEM-FCS, 8–20% of the cells were A2B5⁺, GFAP⁺ type 2 astrocytes, while only 0.5–3% of the cells were

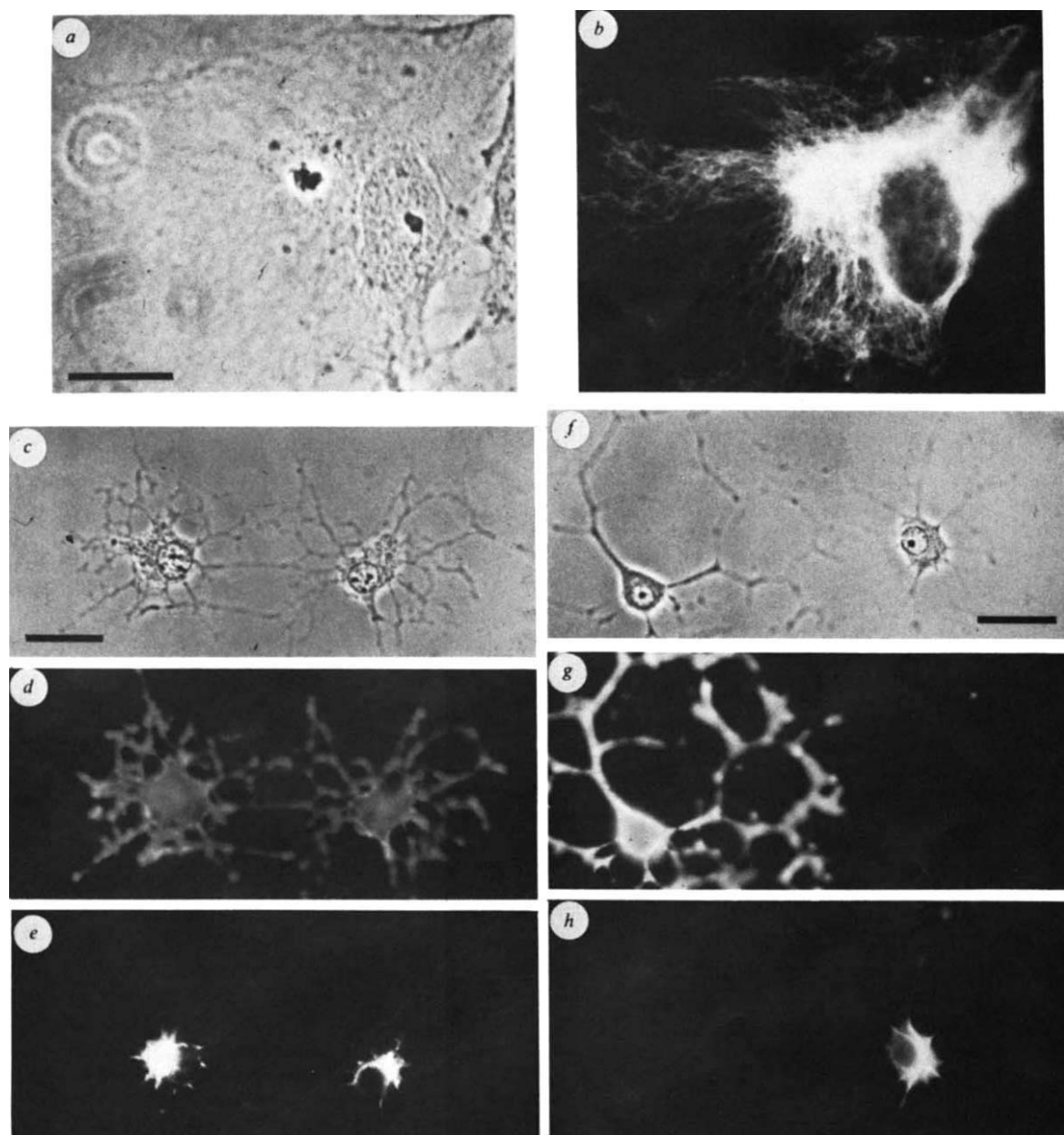


Fig. 1 Three different types of glial cells in cultures of 7-day optic nerve growing on PLL-coated glass coverslips in DMEM-FCS. A type 1 astrocyte is shown in *a* and *b*, two type 2 astrocytes in *c-e* and an oligodendrocyte and a type 2 astrocyte in *f-h*. The cells were labelled after 3 days; those in *c-e* with A2B5 antibody and those in *f-h* with monoclonal anti-GC antibody, in both cases followed by G anti-MIg-Rd. In all cases cells were fixed in acid-alcohol, labelled with rabbit anti-GFAP antiserum followed by G anti-RIg-FI, and then photographed using phase contrast (*a*, *c*, *f*), rhodamine (*d*, *g*) or fluorescein (*b*, *e*, *h*) optics. The fluorescent conjugates were absorbed with Rlg or MIg coupled to Sepharose 6B to remove unwanted cross-reacting antibodies. Note that the type 2 astrocytes are A2B5⁺ (*d*), GFAP⁺ (*e*, *h*) and GC⁻ (*g*), while the oligodendrocyte is GC⁺ (*g*) and GFAP⁻ (*h*). Scale bars, 20 μ m.

GC⁺ oligodendrocytes. However, in serum-free B-S medium only 0.1–2% of the cells were type 2 astrocytes, while 20–40% were oligodendrocytes (Table 1). The oligodendrocytes in serum-free medium usually had more processes than did those in DMEM-FCS and the processes were more highly branched (not shown). As can be seen in Table 1, the critical difference between the two culture conditions was the presence or absence of FCS, rather than the additives in the B-S medium: cultures grown in serum-free DMEM gave results similar to those obtained in serum-free B-S medium, while cultures grown in B-S medium with 10% FCS gave results similar to those obtained with DMEM-FCS. However, results in serum-free B-S medium were more reproducible than those in serum-free DMEM, as the additives enhanced cell survival and oligodendrocyte development (and/or survival) (Table 1).

Several different batches of FCS (from Flow Laboratories and Gibco Bio-cult) gave indistinguishable results when tested

in 7-day optic nerve cultures. Although we have not attempted to characterize in detail the relevant factor(s) in FCS, we have found that it is non-dialysable, is inactivated by boiling for 10 min, precipitates in 60% (v/v) saturated (NH₄)₂SO₄ and is present in delipidated and fibronectin-free FCS (Table 1). FCS also had a striking influence on glial cell development in cultures of 1-day optic nerve, but had somewhat less effect in cultures of 14-day optic nerve (Table 1). Horse serum had relatively little influence on glial cell development in cultures of 7-day optic nerve, while calf serum gave results that were intermediate between those for horse serum and FCS (Table 1). Cell survival in rat serum was usually too poor to obtain meaningful data, but in experiments where cell survival was good, 7-day rat serum gave results similar to those with FCS (not shown).

The effects of FCS on the development of type 2 astrocytes and oligodendrocytes may be interpreted in at least two ways: One is to assume that the two different kinds of glial cell develop

Table 1 Effect of serum on the development of type 2 astrocytes and oligodendrocytes in optic nerve cultures

Age of optic nerve (days)	Culture medium*	No. of cells after 3 days in culture	% Of type 2 astrocytes	% Of oligodendrocytes
7	DMEM+FCS	13,900±1,275	15±2	0.8±0.4
7	DMEM, serum-free	5,200±870	0.6±0.4	15±4
7	B-S medium+FCS	14,105±900	14±2	3±1
7	B-S medium, serum-free	9,405±1,420	1.5±0.6	25±3
7	B-S medium+dialysed FCS	14,660±1,360	14±2	0.7±0.2
7	B-S medium+boiled FCS	10,700±1,100	2±1	23±3
7	B-S medium+60% saturated (NH ₄) ₂ SO ₄ precipitate of FCS	15,250±800	16±3	1±1
7	B-S medium+delipidated FCS	13,400±1,220	17±1	0.7±0.5
7	B-S medium+fibrinectin-free FCS	13,200±1,915	16±1	0.9±0.4
1	DMEM+FCS	15,250±1,455	5±2	0.1±0.1
1	B-S medium, serum-free	8,865±820	0.5±0.3	6±2
14	DMEM+FCS	9,850±1,010	16±3	20±2
14	B-S medium, serum-free	6,870±925	4±3	39±7
7	B-S medium+horse serum	14,650±1,260	4±1	19±3
7	B-S medium+calf serum	11,800±2,600	10±3	12±2

Approximately 30,000 cells were plated in 50 µl of DMEM-FCS in the centre of PLL-coated 35-mm Nunc tissue culture dishes and 2 ml of the appropriate medium were added after 1–2 h. After 3 days the cells were removed with trypsin-EDTA, counted in a haemocytometer (in the presence of trypan blue) and labelled in suspension with A2B5 or anti-GC monoclonal antibodies followed by G anti-MIg-Rd as previously described⁵. After washing, the cells were allowed to adhere to PLL-coated glass coverslips for 35 min at room temperature, and were then fixed in acid-alcohol and labelled with rabbit anti-GFAP followed by G anti-Rlg-FI. The coverslips were mounted in glycerol and the proportion of type 2 astrocytes (A2B5⁺, GFAP⁺ cells) and oligodendrocytes (GC⁺ cells) was determined by counting ≥200 cells in a Zeiss Universal microscope equipped with phase contrast and epi-UV illumination and selective filters for fluorescein and rhodamine, using a ×63 objective. The results in this and the following tables are expressed as the mean ± s.d. of at least three separate experiments.

* Sera were added to a final concentration of 10% (v/v). The 60% (v/v) saturated (NH₄)₂SO₄ precipitate of FCS was made up to the original volume of FCS in saline and dialysed exhaustively against phosphate-buffered saline, pH 7.2. FCS was delipidated with butanol and diisopropyl ether as described elsewhere³⁸. Fibrinectin-free FCS was prepared by passing FCS through a column of Sepharose 4B coupled to gelatin as previously described³⁹. B-S medium consisted of DMEM with added glucose (5.6 mg ml⁻¹), bovine insulin (0.5 µg ml⁻¹), human transferrin (100 µg ml⁻¹), bovine serum albumin (100 µg ml⁻¹), progesterone (0.06 ng ml⁻¹), putrescine (16 µg ml⁻¹), selenium (0.04 ng ml⁻¹), thyroxine (0.4 ng ml⁻¹) and triiodothyronine (0.3 ng ml⁻¹).

Table 2 Effects of killing A2B5⁺ cells or GC⁺ cells on the development of type 2 astrocytes and oligodendrocytes in 7-day optic nerve cultures

Treatment of cells	Culture medium	No. of cells after 3 days in culture	% Of type 2 astrocytes	% Of oligodendrocytes
Normal ascites fluid+complement	DMEM-FCS	9,755±1,050	13±2	1±0.5
	Serum-free B-S medium	6,350±1,345	0.9±1	31±4
A2B5 antibody+complement	DMEM-FCS	7,540±850	0	0
	Serum-free B-S medium	3,820±1,250	0	0
Anti-GC antibody+complement	DMEM-FCS	10,500±865	10±3	0.8±3
	Serum-free B-S medium	6,900±1,100	0.6±0.5	24±3

Approximately 40,000 7-day optic nerve cells were incubated in 200 µl of A2B5 or anti-GC monoclonal antibody, or normal ascites fluid together with agarose-absorbed rabbit complement for 40 min at 37 °C as previously described⁵. The final dilutions were antibodies and normal ascites, 1:50 and complement, 1:10. After washing, the number of living cells was determined and the cells were cultured in PLL-coated, 35-mm Nunc dishes. The number of living cells cultured per dish after treatment with normal ascites fluid, A2B5 antibody and anti-GC antibody plus complement were 15,300±2,300, 6,250±1,750 and 13,165±2,100, respectively. After 3 days the cells were removed from the dish, counted and labelled as in Table 1.

from two distinct precursor cells and that FCS favours the development and/or survival of type 2 astrocytes while inhibiting the development and/or survival of oligodendrocytes. The other is to assume that both kinds of glial cell develop from a single type of progenitor cell, which develops mainly into type 2 astrocytes in the presence of FCS and mainly into oligodendrocytes in its absence. We provide several lines of evidence to suggest that the latter interpretation is correct.

A2B5⁺ precursor cells

We have previously used two strategies to demonstrate that most type 2 astrocytes in optic nerve cultures develop from A2B5⁺ precursor cells⁵: (1) if 7-day optic nerve cells are killed with A2B5 antibody and complement before culture, no type 2 astrocytes develop; (2) if 7-day optic nerve cells are prelabeled with A2B5 antibody before culture, 65–75% of the type 2 astrocytes that develop after 2 days have residual A2B5 antibody on their surface, indicating that they have developed from A2B5⁺ cells in the initial cell suspension. In the present study,

we used the same two strategies to demonstrate that the great majority of oligodendrocytes in cultures of 7-day optic nerve also develop from A2B5⁺ precursor cells.

When 7-day optic nerve cells were treated with A2B5 antibody and rabbit complement and then cultured in DMEM-FCS or in serum-free B-S medium, neither type 2 astrocytes nor oligodendrocytes developed (Table 2). In addition, as found previously⁵, no A2B5⁺ cells were seen in these cultures, suggesting that A2B5⁺ cells do not develop into A2B5⁺ cells. On the other hand, the numbers of type 1 astrocytes (A2B5⁻, GFAP⁺ cells) were not significantly different from those in cultures treated with complement alone (not shown; see also ref. 5), suggesting that A2B5⁺ cells do not develop into type 1 astrocytes in 7-day optic nerve cultures.

Although we were able to kill more than 95% of the GC⁺ cells in optic nerve cultures by treating the cultures with monoclonal anti-GC antibody and rabbit complement (not shown), treating 7-day optic nerve cells in suspension with this antibody and complement before culture had little effect on the numbers

of oligodendrocytes or type 2 astrocytes seen after 3 days in culture (Table 2). This suggests that the great majority of these glial cells developed in culture from GC⁺ precursor cells.

While the antibody and complement-mediated killing experiments indicate that A2B5⁺ cells are required for the development of both oligodendrocytes and type 2 astrocytes, they do not establish that the oligodendrocyte precursor cells themselves are A2B5⁺. To determine this directly, we treated 7-day optic nerve cells with monoclonal anti-GC antibody and rabbit complement to kill the existing oligodendrocytes, then incubated the cells in A2B5 (IgM) antibody for 40 min, and, after thorough washing, cultured them in serum-free B-S medium on PLL-coated glass coverslips. After 48 h, the cells were labelled with class-specific G anti-IgM-Rd (Nordic, 1:40) to detect residual A2B5 antibody, followed by monoclonal anti-GC antibody (IgG3) and then class-specific G anti-IgG3-Fl (Nordic, 1:80) to identify the newly formed oligodendrocytes. In such experiments, more than 90% of the GC⁺ oligodendrocytes had residual A2B5 antibody on their surface, indicating that they had developed from A2B5⁺ cells.

In a separate study, we have used fluorescence-activated cell sorting to show that oligodendrocyte precursors in 14-day embryonic rat brain are A2B5⁺; in addition, we found that newly formed GC⁺ oligodendrocytes in neonatal corpus callosum are A2B5⁺, but become A2B5⁻ as they mature¹³. This is also the case in optic nerve: when 7-day optic nerve cells were cultured in serum-free B-S medium and double-labelled with A2B5 and anti-GC monoclonal antibodies followed by immunoglobulin-class-specific fluorescent conjugates, more than 95% of the GC⁺ cells were A2B5⁺ after 3 days in culture, but less than 50% were A2B5⁺ after 7 days in culture (not shown).

A common progenitor cell

The evidence that both type 2 astrocytes and oligodendrocytes develop from A2B5⁺ cells in optic nerve cultures is consistent with the possibility that both types of glial cell develop from a common progenitor, but it does not exclude the possibility that there are two distinct precursor cells, both of which are A2B5⁺. To distinguish between these two possibilities we examined the development of glial cells in optic nerve cultures more quantitatively. We cultured a known number of optic nerve cells in DMEM-FCS or in serum-free B-S medium, counted the total number of cells that adhered to the culture dish after 18 h and determined their antigenic phenotypes. After 72 h, we counted the total numbers of type 2 astrocytes and oligodendrocytes that developed in parallel cultures. We also exposed parallel cultures to ³H-thymidine from 18 h onward and determined (by indirect immunofluorescence followed by autoradiography) the proportion of type 2 astrocytes and oligodendrocytes (or their precursors) that had synthesized DNA during this period.

As can be seen in Tables 3 and 4, the A2B5⁺ cells (which include all of the type 2 astrocytes (by definition) and more than 95% of the GC⁺ oligodendrocytes, as well as the precursors for both of these glial cells (see above)) divided very little during these experiments. Less than 25% of these cells incorporated detectable amounts of ³H-thymidine when cultured in FCS and

Table 3 Incorporation of ³H-thymidine into developing type 2 astrocytes and oligodendrocytes in 7-day optic nerve cultures studied by autoradiography

Culture medium	% Of type 2 astrocytes radiolabelled	% Of oligodendrocytes radiolabelled	% Of A2B5 ⁺ cells radiolabelled
DMEM-FCS	24 ± 3	<1	22 ± 4
Serum-free B-S medium	<1	<1	<1

Approximately 20,000 cells were cultured on PLL-coated Nunc dishes. ³H-thymidine (2 Ci mmol⁻¹, Amersham) was added at 2 µCi ml⁻¹ after 18 h and again at 48 h. After 72 h the cells were removed from the dish and labelled as in Table 1. The coverslips were coated with Ilford K2 emulsion, stored at -70°C for 72 h in the dark, developed with Ilford Super Contrast FF, mounted and examined as described above. Cells with >10 grains over their nuclei were scored as radiolabelled. The numbers of cells at the end of 72 h were not significantly different from those in cultures without added ³H-thymidine.

less than 1% of them did so in serum-free medium (Table 3). Moreover, the number of A2B5⁺ cells remained approximately constant from 18 to 72 h (Table 4). However, the numbers of A2B5⁺ cells that were GFAP⁺ (that is, type 2 astrocytes) dramatically increased between 18 and 72 h in FCS-containing cultures, as did the numbers of A2B5⁺ cells that were GC⁺ (that is, oligodendrocytes) in serum-free cultures. In fact, the numbers of type 2 astrocytes that developed in serum, or of oligodendrocytes that developed in the absence of serum, were always more than 70–80% of the total number of A2B5⁺ cells that adhered to the culture dish at 18 h, even though most of these cells did not divide during the culture period. In the best experiments (Table 4), in serum-free medium almost all of the A2B5⁺ cells developed into oligodendrocytes, apparently without synthesizing DNA (Table 3), whereas in FCS most of them developed into type 2 astrocytes. It is difficult to escape the conclusion that a large proportion of the A2B5⁺ cells in 7-day optic nerve can develop either into type 2 astrocytes or into oligodendrocytes, depending on the presence or absence of FCS.

Co-expression of GFAP and galactocerebroside

As GFAP and GC are widely used as specific markers for astrocytes and oligodendrocytes, respectively, in CNS cultures^{4,11,14,15}, we were surprised to find some cells in optic nerve cultures that expressed both molecules. Such GC⁺, GFAP⁺ cells were uncommon in cultures grown in serum-free medium, but they were more frequent in cultures grown in B-S medium containing FCS. If 7-day optic nerve cells were cultured for 2 days in the latter medium and then switched to serum-free B-S medium for 2 days, up to 80% of the process-bearing GFAP⁺ cells were GC⁺. While most GC⁺, GFAP⁺ cells were labelled brightly with anti-GC antibody and weakly with anti-GFAP antiserum, or vice versa, some cells were labelled intensely with both antibodies (Fig. 2). The same results were obtained with

Table 4 Numbers of type 2 astrocytes and oligodendrocytes that develop from a known number of A2B5⁺ cells in cultures of 7-day optic nerve

Hours in culture	Culture medium	Total cells (cells per dish)	Total A2B5 ⁺ cells* (cells per dish)	Type 2 astrocytes (cells per dish)	Oligodendrocytes (cells per dish)
18	DMEM-FCS	4,560 ± 260	2,025 ± 110	62 ± 5	387 ± 11
18	Serum-free B-S medium	4,850 ± 490	2,250 ± 185	29 ± 6	554 ± 13
72	DMEM-FCS	14,600 ± 1,515	1,950 ± 210	1,820 ± 98	135 ± 12
72	Serum-free B-S medium	7,100 ± 285	2,130 ± 165	188 ± 8	1,918 ± 114

Approximately 30,000 cells were plated on PLL-coated culture dishes. Half of the dishes were studied after 18 h, and half after 72 h. The cells were removed, counted and labelled as in Table 1. The number of type 2 astrocytes and oligodendrocytes per dish was determined by multiplying the proportion of these cell types by the total numbers of cells in the dish.

* This population includes all of the type 2 astrocytes (A2B5⁺, GFAP⁺ cells) and almost all of the oligodendrocytes (GC⁺ cells, of which >95% are also A2B5⁺, see text).

two different rabbit anti-GFAP sera (one made by Pruss¹⁶ and the other by Dahl and Bignami¹⁷) and with two different monoclonal anti-GC antibodies (one made by Ranscht *et al.*¹² and the other (01) by Sommer and Schachner¹⁸). Moreover, when 7-day optic nerve cells growing in B-S medium containing 10% FCS were studied by immunogold electron microscopy (see below), some cells with glial filaments were labelled with monoclonal anti-GC antibody (not shown). The number of GC⁺, GFAP⁺ cells in cultures of 14-day optic nerve were always less than in cultures of 7-day optic nerve.

To determine whether such glial cells with a mixed astrocyte-oligodendrocyte phenotype were present in normal CNS tissue, we examined cell suspensions from freshly dissected 7–14-day optic nerve or corpus callosum as previously described⁵. In these experiments we found many A2B5⁺, GFAP⁺ cells (as previously reported⁵), but no convincing examples of GC⁺, GFAP⁺ cells, suggesting that this mixed phenotype results from aberrant differentiation induced by the culture conditions and does not represent a normal stage in the development of oligodendrocytes or fibrous astrocytes. Nonetheless, the finding of GC⁺, GFAP⁺ cells in culture serves to emphasize the close developmental relationship between these two types of glial cell and supports our conclusion that they both develop from a common progenitor cell.

Are type 2 astrocytes artefacts?

Quantitative morphological and autoradiographic studies of glial cell development in the rat optic nerve^{19–21} suggest that, *in vivo*, most of the A2B5⁺ glial cell precursors in 7-day optic nerve normally develop into oligodendrocytes. Yet, when cultured in the presence of FCS, almost all of these cells were induced to become type 2 astrocytes (Table 4). Is it possible then that type 2 astrocytes are a tissue culture artefact—oligodendrocyte precursors gone wrong *in vitro*? Two lines of evidence suggest that this is not the case.

First, we have previously shown that cells with the antigenic phenotype of type 2 astrocytes (A2B5⁺, GFAP⁺) are present in freshly dissociated neonatal white matter but not grey matter⁵. Moreover, in frozen sections of adult CNS, most astrocytes in white matter, but not in grey matter, are A2B5⁺ (R.H.M. and M.C.R., in preparation). Thus, type 2 astrocytes are probably fibrous astrocytes and are found *in vivo* as well as *in vitro*.

Second, although most of the type 2 astrocytes that develop in culture in the presence of FCS look more like oligodendrocytes than type 1 astrocytes by light microscopy, when studied by immunogold electron microscopy, most had the characteristic features of astrocytes²²: they contained glial filaments (single and in bundles), elongated mitochondria, dense bodies and smaller dense structures resembling glycogen granules, all in a light, granular cytoplasm; some also contained many microtubules, characteristic of immature astrocytes (Fig. 3a, b). On the other hand, most GC⁺ oligodendrocytes that developed in serum-free B-S medium contained no detectable glial filaments, dense bodies, or glycogen granules, but had more microtubules and usually a darker cytoplasm than the type 2 astrocytes (Fig. 3c, d). In addition, type 2 astrocytes shared another property with type 1 astrocytes: they could be labelled intracellularly by a rabbit antiserum against glutamine synthetase (provided by Ole Jørgensen and used at 1:40 on acid-alcohol-fixed cells). Glutamine synthetase has been previously found in fibrous and protoplasmic astrocytes²³, as well as in retinal Müller cells^{24–26}, but not in oligodendrocytes or CNS neurones. In the present studies, anti-glutamine synthetase antiserum labelled more than 90% of both type 1 and type 2 astrocytes but did not label oligodendrocytes in optic nerve cultures grown in either DMEM-FCS or serum-free B-S medium (not shown).

Implications

By combining the use of tissue culture and antibodies we have identified a cell type in 7-day optic nerve that develops *in vitro* into a fibrous astrocyte or an oligodendrocyte, depending on

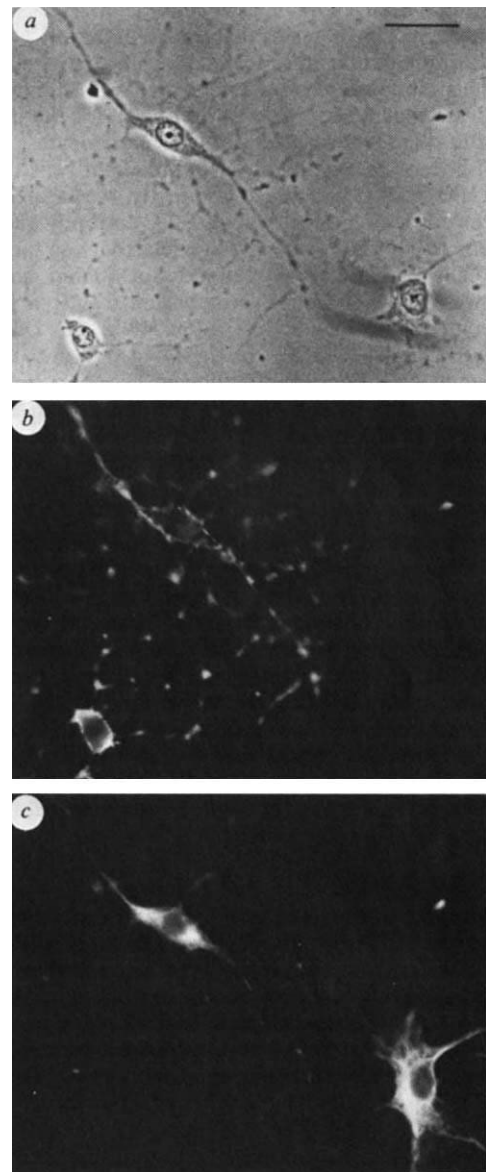


Fig. 2 A GC⁺, GFAP⁺ cell, a GC⁺, GFAP⁺ type 2 astrocyte and a GC⁺, GFAP⁺ oligodendrocyte in a culture of 7-day optic nerve growing on a PLL-coated glass coverslip in B-S medium with 10% FCS. After 3 days in culture, the cells were labelled and photographed as in *f–h* in Fig. 1. The fact that the type 2 astrocyte is unlabelled by the anti-GC antibody (*b*) while the oligodendrocyte is unlabelled by the anti-GFAP antiserum (*c*) attests to the specificity of these reagents and the fluorescent conjugates. Scale bar, 20 μ m.

whether or not the culture medium contains FCS. Even in 14-day rats, the oldest studied, the optic nerve seemed to contain cells that could be directed along one glial differentiation pathway or the other. While one must be cautious in extrapolating from development in culture to development in the animal (as some of our own results emphasize), it seems likely that fibrous astrocytes and oligodendrocytes develop from a common progenitor cell *in vivo* just as they do *in vitro*. The neoplastic transformation of such progenitor cells could explain the occurrence of mixed astrocyte-oligodendrocyte tumours in man²⁷. The close developmental relationship between fibrous astrocytes and oligodendrocytes may mirror a close phylogenetic relationship. For example, in the optic nerve of newts, a single type of glial cell functions as both an astrocyte (having glial filaments and forming the glial limiting membrane) and

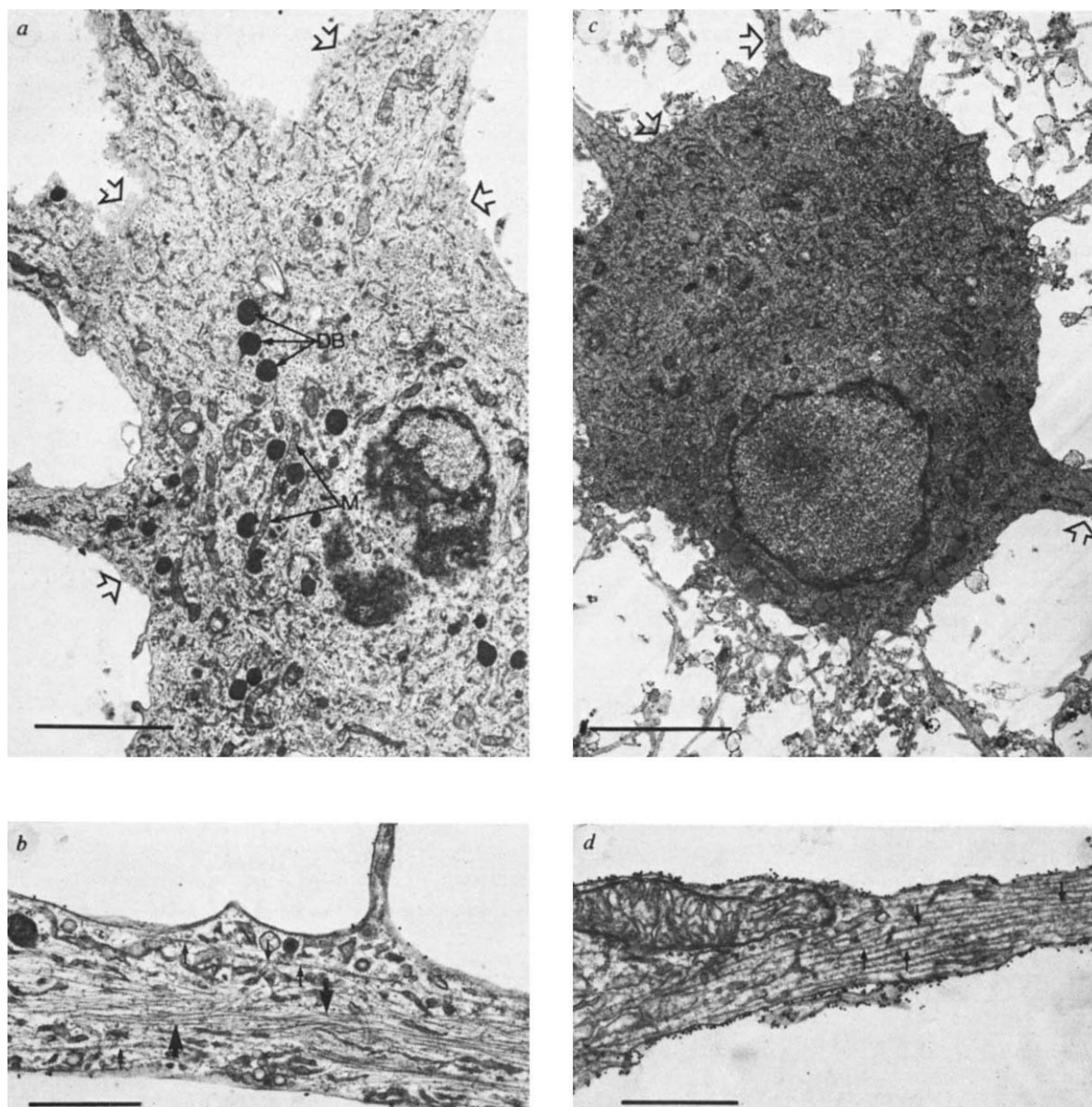


Fig. 3 Electron micrographs of the cell body (*a, c*) and a process (*b, d*) of a type 2 astrocyte (*a, b*) and an oligodendrocyte (*c, d*) from cultures of 7-day optic nerve growing on PLL-coated culture dish in DMEM-FCS (*a, b*) or serum-free B-S medium (*c, d*). The cultures were prefixed in 1% glutaraldehyde for 20 min, incubated with A2B5 (*a, b*) or monoclonal anti-GC antibody (*c, d*) for 30 min followed by Ganti-Mlg-gold (Janssen, 1:5) for 30 min, post-fixed for 20 min in 2% glutaraldehyde, osmicated and stained with 0.5% aqueous uranyl acetate, dehydrated through graded ethanol and embedded in Epon 812 (EM Scope). Thin sections were cut parallel to the substrate and stained with uranyl acetate and lead citrate before examination on a Jeol 100 CXII electron microscope. The gold-coupled antibody (open arrows) is just visible at low magnification (*a, c*) but is readily seen at higher magnification (*b, d*). The labelling of type 2 astrocytes with A2B5 antibody (*a, b*) is concentrated on the cell body, with relatively little on the cell processes, while the labelling of the oligodendrocyte with anti-GC antibody has the opposite distribution (*c, d*). Note the dense bodies (DB), elongated mitochondria (M) and glial filaments (large arrows in *b*) in the astrocyte and the large number of microtubules (small arrows) and absence of glial filaments in the oligodendrocyte. Approximately 40 cells of each type were examined and more than 80% had most of the characteristic features of their class. When monoclonal antibodies were omitted from the staining procedure no labelling was seen. Scale bars, 5 μ m in *a, c*; 1 μ m in *b, d*.

an oligodendrocyte (myelinating axons)²⁸, while in the newt spinal cord²⁹ and in higher vertebrates these two functions are performed by two morphologically distinct cell types.

Our demonstration of developmental plasticity and environmental influence in the differentiation of CNS glial cells *in vitro* is in some respects comparable to observations made on autonomic neurones in the peripheral nervous system. In cultures of neonatal rat superior cervical ganglion, developing sympathetic neurones have either adrenergic or cholinergic properties depending on the culture conditions³⁰. Moreover, some superior cervical ganglion neurones in culture have a mixed adrenergic-cholinergic phenotype, secreting both noradrenaline and acetylcholine³¹, just as we have found some cells in optic nerve cultures with a mixed astrocyte-oligoden-

drocyte phenotype expressing both GFAP and GC. However, while cholinergic sympathetic neurones pass through an adrenergic phase during their development *in vitro*³¹, and probably *in vivo*³², we have found no evidence that oligodendrocytes make GFAP or that type 2 astrocytes make GC during their normal development.

It is perhaps more instructive to compare our findings with results obtained in studies of haematopoiesis, where a single type of pluripotent stem cell gives rise to a large number of different types of specialized blood cells. Blood cell differentiation proceeds by a series of steps in which progenitor cells become progressively restricted in their choice of differentiation pathway³³. For example, one type of progenitor cell has been defined that can develop into either a neutrophilic granulocyte

or a macrophage, but not into any other kind of blood cell; the decision to become a granulocyte or a macrophage seems to depend on the concentration of a glycoprotein signalling molecule, granulocyte/macrophage colony-stimulating factor (GM-CSF)³⁴. As neural progenitor cells in developing optic nerve seem not to differentiate into neurones or ependymal cells *in vivo*, and as we have found no evidence that they can develop into neurofilament-containing neurones or type 1 astrocytes *in vitro*⁵, our results are consistent with the hypothesis that the A2B5⁺ glial progenitor cell in 7-day optic nerve is committed to becoming either an oligodendrocyte or a type 2 (fibrous) astrocyte.

But what determines which of these differentiation pathways such a glial progenitor cell will follow? While it is likely that signals from axons normally have an important influence on the decision *in vivo*³⁵, it is clear that these cells can be induced to develop into either fibrous astrocytes or oligodendrocytes *in vitro* in the absence of recognizable neurones. Perhaps an

intimate association with axons permits a cell to become an oligodendrocyte by protecting it from a soluble signal (such as the one in perinatal serum) that would otherwise induce it to become an astrocyte. Or perhaps degenerating axons (resulting from the death of retinal ganglion cells that occurs during normal development^{36,37}) induce neighbouring progenitor cells to become fibrous astrocytes rather than oligodendrocytes. Since the decision can be controlled in culture it should be possible to determine the cellular and molecular mechanisms that underlie it.

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- Jacobson, M. *Developmental Neurobiology* 2nd edn, 27–55 (Plenum, New York, 1978).
- Bignami, A., Eng, L. F., Dahl, D. & Uyeda, C. T. *Brain Res.* **43**, 429–435 (1972).
- Schachner, M. *et al. J. Cell Biol.* **75**, 67–73 (1977).
- Raff, M. C. *et al. Brain Res.* **174**, 283–308 (1979).
- Raff, M. C. *et al. J. Neurosci.* (in the press).
- Van Heyningen, W. E. *J. gen. Microbiol.* **31**, 375–387 (1963).
- Eisenbarth, G. S., Walsh, F. S. & Nirenberg, M. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4913–4917 (1979).
- Dimpfel, W., Huang, R. T. C. & Habermann, E. *J. Neurochem.* **29**, 329–334 (1977).
- Mirsky, R. *et al. Brain Res.* **148**, 251–259 (1978).
- Bottenstein, J. E. & Sato, G. H. *Proc. natn. Acad. Sci. U.S.A.* **76**, 514–517 (1979).
- Raff, M. C. *et al. Nature* **274**, 813–816 (1978).
- Ranscht, B. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 2709–2713 (1982).
- Abney, E., Williams, B. & Raff, M. C. *Dev. Biol.* (submitted).
- Mirsky, R. in *Neuroimmunology* (ed. Brookes, J.) 141–181 (Plenum, New York, 1982).
- Schachner, M. in *Neuroimmunology* (ed. Brookes, J.) 215–250 (Plenum, New York, 1982).
- Pruss, R. *Nature* **280**, 688–690 (1979).
- Dahl, D. & Bignami, A. *Brain Res.* **116**, 150–157 (1976).
- Sommer, I. & Schachner, M. *Dev. Biol.* **83**, 311–327 (1981).
- Vaughn, J. Z. *Zellforsch.* **94**, 293–324 (1969).
- Skoff, R. P., Price, D. L. & Stocks, A. J. *comp. Neurol.* **169**, 291–312 (1976).
- Skoff, R. P., Price, D. L. & Stocks, A. J. *comp. Neurol.* **169**, 313–333 (1976).
- Peters, A., Palay, S. L. & Webster, H. de F. in *The Fine Structure of the Nervous System: The Neurons and Supporting Cells* 2nd edn, 233–244 (Saunders, Philadelphia, 1976).
- Norenberg, M. D. & Martinez-Hernandez, A. *Brain Res.* **161**, 303–310 (1979).
- Riepe, R. E. & Norenberg, M. D. *Nature* **268**, 654–655 (1977).
- Moscona, A. A., Mayerson, P., Linser, P. & Moscona, M. in *Tissue Culture in Neurobiology* (eds Giacobini, E., Vernadakis, A. & Shahr, A.) 111–127 (Raven, New York, 1980).
- Sarthy, P. V. & Lam, D. M. K. *J. Cell Biol.* **78**, 675–684 (1978).
- Russell, D. S. & Rubinstein, L. J. in *Pathology of Tumours of the Nervous System*, 127–131 (Arnold, London, 1959).
- Turner, J. E. & Singer, M. J. *comp. Neurol.* **156**, 1–18 (1974).
- Schonbach, C. J. *comp. Neurol.* **135**, 93–119 (1969).
- Patterson, P. H. A. *Rev. Neurosci.* **1**, 1–17 (1978).
- Furshpan, E. J., MacLeish, P. R., O'Leary, P. H. & Potter, D. D. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4225–4229 (1976).
- Landis, S. C. & Patterson, P. H. *Trends Neurosci.* **4**, 172–175 (1981).
- Metcalf, D. & Moore, M. A. S. *Haemopoietic Cells* (North-Holland, Amsterdam, 1971).
- Metcalf, D. in *Cellular Controls in Differentiation* (eds Lloyd, C. W. & Rees, D. A.) 125–144 (Academic, London, 1981).
- Privat, A., Valat, J. & Fulcrand, J. J. *Neuropath. exp. Neurol.* **40**, 46–60 (1981).
- Potts, R., Dreher, B. & Bennett, M. R. *Dev. Brain Res.* **3**, 481–486 (1982).
- Lam, K., Sefton, A. J. & Bennett, M. R. *Dev. Brain Res.* **3**, 487–491 (1982).
- Cham, B. E. & Knowles, B. R. *J. Lipid Res.* **17**, 176–181 (1976).
- Engvall, E. & Ruoslahti, E. *Int. J. Cancer* **20**, 1–5 (1977).

Identification of transforming gene in two human sarcoma cell lines as a new member of the *ras* gene family located on chromosome 1

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*A molecular clone containing part of the transforming gene from two human sarcoma cell lines, HT1080 and RD, has been obtained and shown to represent a new member of the human *ras* gene family. The transforming gene has undergone no major rearrangements and has not been amplified in either sarcoma cell line. The major transcript from the gene is 2,200 nucleotides long and is present at the same levels in both normal fibroblasts and tumour cells. The same gene is also activated in HL60, a promyelocytic leukaemia line and in SK-N-SH, a neuroblastoma line. The gene, N-ras, is located on chromosome 1.*

DNA TRANSFECTION studies using NIH/3T3 cells as recipients have led to the identification of different transforming genes present in a variety of human tumour cell lines^{1–5}. In the case of the human bladder tumour line, EJ, the transforming gene has been cloned and shown to be an activated form of the c-Ha-ras1 gene^{6–10}, which is one of two distinct cellular sequences¹¹ that have been shown to share homology with v-Ha-ras, the oncogene carried by Harvey murine sarcoma virus¹². The activation of this gene is the result of an alteration in the coding sequence of the cellular p21 protein as a con-

sequence of a single base mutation^{13–15}. Another *ras* transforming gene has been identified in a wide variety of different tumour cell lines (for example, colon, lung, pancreas, bladder and a rhabdomyosarcoma)^{5,10}, and has been shown to be an activated form of c-Ki-ras2 (one of the two known cellular homologues¹¹ of v-Ki-ras, the viral oncogene carried by Kirsten sarcoma virus¹⁶), though in these cases the mechanism of activation is not known¹⁷. It has now been shown that more than two-thirds of all solid human tumours or cell lines, except lymphocyte neoplasms³, having a detectable transforming gene contain an

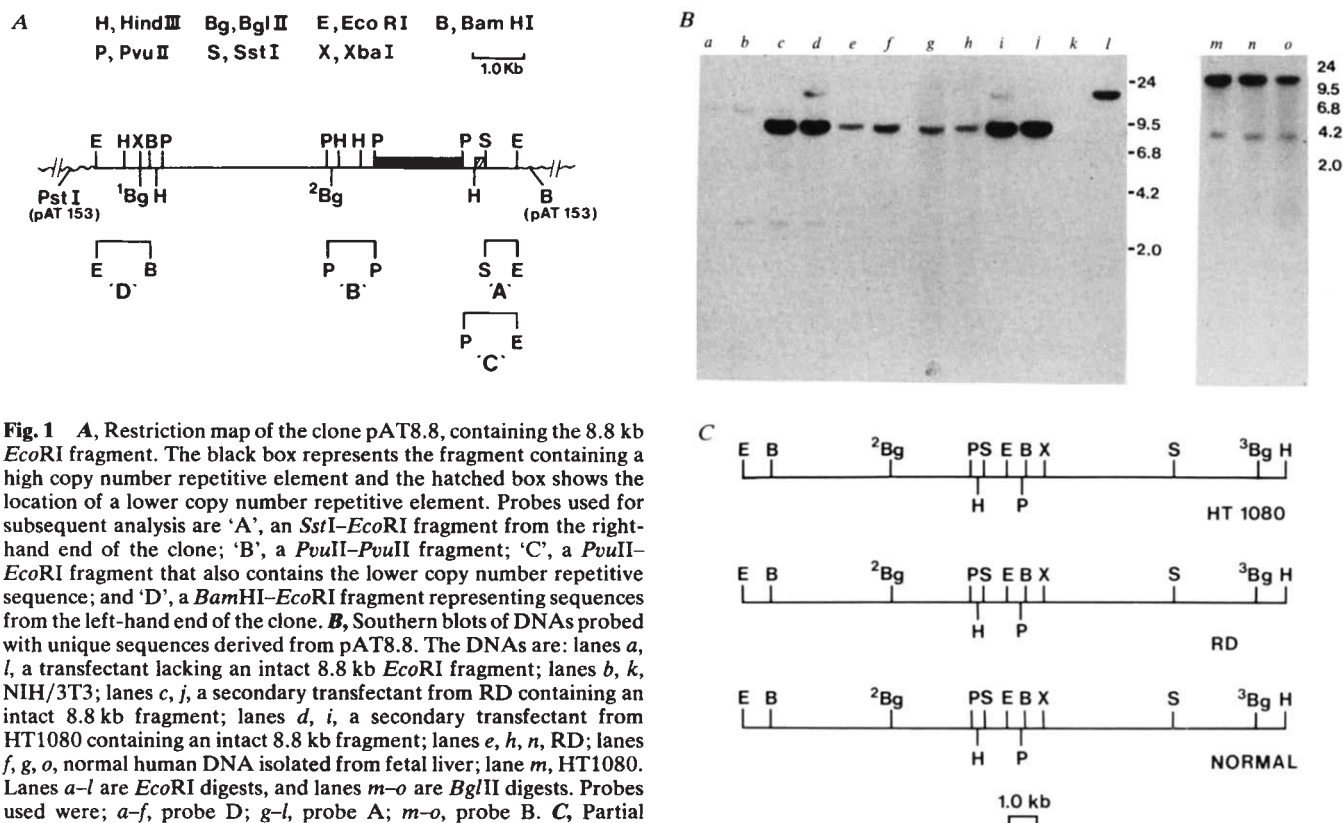


Fig. 1 **A**, Restriction map of the clone pAT8.8, containing the 8.8 kb *EcoRI* fragment. The black box represents the fragment containing a high copy number repetitive element and the hatched box shows the location of a lower copy number repetitive element. Probes used for subsequent analysis are 'A', an *SstI*-*EcoRI* fragment from the right-hand end of the clone; 'B', a *PvuII*-*PvuII* fragment; 'C', a *PvuII*-*EcoRI* fragment that also contains the lower copy number repetitive sequence; and 'D', a *BamHI*-*EcoRI* fragment representing sequences from the left-hand end of the clone. **B**, Southern blots of DNAs probed with unique sequences derived from pAT8.8. The DNAs are: lanes a, l, a transfectant lacking an intact 8.8 kb *EcoRI* fragment; lanes b, k, NIH/3T3; lanes c, j, a secondary transfectant from RD containing an intact 8.8 kb fragment; lanes d, i, a secondary transfectant from HT1080 containing an intact 8.8 kb fragment; lanes e, h, n, RD; lanes f, g, o, normal human DNA isolated from fetal liver; lane m, HT1080. Lanes a-l are *EcoRI* digests, and lanes m-o are *BglII* digests. Probes used were; a-f, probe D; g-l, probe A; m-o, probe B. **C**, Partial restriction maps of the transforming gene in HT1080 and RD and its counterpart in normal human DNA.

Methods: **A**, DNA was isolated from an HT1080 secondary transfectant that contained only three *EcoRI* fragments (one of them being the 8.8 kb fragment) hybridizing to a nick-translated total human DNA probe. The DNA (200 µg) was digested with *EcoRI*, size-fractionated on a 5–20% potassium acetate gradient, and the fractions containing the 8.8 kb fragment were identified after analysing a small aliquot from each on a Southern blot. DNA from enriched fractions was ligated with the arms of *EcoRI* digested Charon 4A phage DNA and the mixture was packaged *in vitro*. A library containing 2×10^5 recombinants was screened using a total human DNA probe and four recombinants were identified that contained a human repetitive sequence. Analysis of one of these clones confirmed the presence of an 8.8 kb *EcoRI* fragment. The recombinant phage DNA was digested with *EcoRI* and the insert was subcloned into pAT153. A clone was obtained, pAT8.8, which was found to contain an 8.8 kb insert and a restriction map was obtained using standard procedures with single, double and partial enzyme digests. In **B**, transfectants lacking an 8.8 kb *EcoRI* fragment were derived as follows: DNA (600 µg) was isolated from a primary transfectant, partially digested with *Sau3A*, and centrifuged through a 5–20% potassium acetate gradient. Fractions were pooled as follows: pool 1, 5–10 kb; pool 2, 10–16 kb; and pool 3, 16–22 kb. These pooled DNAs (60 µg of each) were used to transfect NIH/3T3 cells and only pools 2 and 3 yielded transformed foci. It was found that transfectants derived using pool 2 DNA contained only the 8.8 kb *EcoRI* fragment harbouring a human repetitive sequence whilst pool 3 transfectants had both an 8.8 kb and a 7.8 kb *EcoRI* fragment. DNA isolated from the latter was then used in a third round of transfection yielding foci that still had the 7.8 kb fragment but no longer contained an intact 8.8 kb species. In **C**, to map restriction sites in RD and normal DNA corresponding to those in the cloned fragment, DNA from all three sources (RD, HT1080, and normal) was digested with the relevant restriction enzymes (or combination of restriction enzymes) and analysed using Southern blots probed with a number of fragments derived from the cloned sequence. To map restriction sites outside of and to the right of the cloned sequence, DNA from HT1080, RD and normal DNA was digested with the relevant enzyme and probed on blots with probe A. This only allows identification of the nearest restriction site to the cloned fragment.

activated form of c-Ki-ras²⁵. Both the Kirsten and Harvey viral oncogenes show extensive homology at the protein and DNA level^{18–20}, and it is also apparent that c-Ha-ras1 and c-Ki-ras2, along with c-Ha-ras2 and c-Ki-ras1, are four members of a human ras gene family¹¹.

We have previously identified a transforming gene in two human sarcoma cell lines, HT1080 (a fibrosarcoma line) and RD (a rhabdomyosarcoma line) and have shown that this gene is neither c-Ha-ras1 nor c-Ki-ras2⁴. Here we report that this transforming gene is a new member of the human ras gene family located on chromosome one.

Molecular cloning

DNAs isolated from second-round NIH/3T3 transfectants derived from both HT1080 or RD contain an 8.8 kilobase (kb) *EcoRI* fragment that carries a human repetitive sequence and we have concluded that this fragment is closely linked to the transforming gene⁴. Accordingly a partial gene library was

constructed in Charon 4A, using DNA isolated from a secondary HT1080 transfectant that had been digested with *EcoRI* and enriched for the 8.8 kb fragment by size fractionation. Recombinant phage carrying a human repetitive sequence were identified after screening the library with nick-translated total human DNA. One recombinant was further characterized and found to contain an 8.8 kb fragment. This was subcloned into pAT153²¹ and a restriction map of the recombinant plasmid, pAT8.8, is shown in Fig. 1A. The clone contains two repetitive elements, as judged by hybridization to a total human DNA probe; one (contained within the black box, Fig. 1A) being present at around 10^5 copies and the other (within the hatched box, Fig. 1A) at around 10^4 copies, in the human genome. Unique sequences derived from the clone hybridized to an 8.8 kb *EcoRI* fragment in transfectants derived from both HT1080 and RD. Isoenzyme analysis has shown that these two human cell lines are different (S. Povey, personal communication), thereby demonstrating that the transforming gene in these two different cell lines is the same.

Boundary mapping

To determine which sequences, if any, within the cloned fragment are not required for biological activity, we used transfectants (derived from DNA that had been partially digested with *Sau3A* before transfection) which no longer show an intact 8.8 kb *EcoRI* fragment when hybridized to a total human DNA probe. *EcoRI* digests of DNA isolated from these transfectants were probed on Southern blots with sequences derived from the left-hand end (probe D, Fig. 1A) or the right-hand end (probe A, Fig. 1A) of the cloned fragment. The results obtained with one such transfectant are shown in Fig. 1B. As expected, both probes hybridize to an 8.8 kb *EcoRI* fragment in normal (lanes f, g) and RD (lanes e, h) DNA, and in HT1080 (lanes d, i) and RD (lanes c, j) transfectants that contain an intact 8.8 kb sequence. However, although probe D does cross-hybridize with some mouse sequences (lane b), no human sequences are contained in the transfectant lacking an intact 8.8 kb fragment (lane a). Probe A, on the other hand, clearly hybridizes to human sequences in this transfectant (lane l). The biologically active transforming gene must, therefore, be to the right of the *BamHI* site in the cloned sequence. All transfectants so far analysed maintain the *BglII* site 2 (Fig. 1A).

Using probe A it was also possible by Southern blot analysis to map several restriction sites in HT1080 DNA located to the right of the cloned fragment. The results are shown in Fig. 1C; in particular there is a *HindIII* site, a *BglII* site and an *SstI* site, 9.0, 8.0 and 5.5 kb, respectively, to the right of the cloned fragment. This same probe A was then used to screen all NIH/3T3 transfectants derived from HT1080 and RD. All maintained the *PvuII* site mapped at approximately 0.5 kb into the uncloned segment (Fig. 1C), but three transfectants no longer had the *SstI* site 5.5 kb away from the cloned sequence. We conclude, therefore, that the oncogene must be to the left of this *SstI* site and is contained within a 13 kb stretch of DNA, 7.8 kb of which is carried by clone pAT8.8.

Comparison with its normal allele

Using a variety of probes derived from pAT8.8 it was possible to compare the restriction map of the transforming gene in HT1080 with that of RD and of DNA isolated from normal human fetal liver (Fig. 1C). No differences could be detected amongst any of the three loci. Furthermore, the intensity of hybridization of cloned probes with HT1080, RD and normal DNA (Fig. 1B, lanes m, n, o) show that there has been no detectable gene amplification in the tumour cell lines. Gene amplification does seem to have occurred in some (Fig. 1B, lanes i, j) transfectants, though we have not been able to correlate gene copy number with any altered biological features of the transfected cells.

RNA from the transforming gene

Figure 2 shows the result of a Northern blot of poly(A) selected mRNA hybridized with probe B (Fig. 1A). Three major species of RNA can be seen, with sizes of 5,800, 2,200 and 1,500 nucleotides, in HT1080 cells (lane c), and in both HT1080 (lane d) and RD (lane e) transfectants. Probe C (Fig. 1A) also gave a pattern of hybridization on Northern blots similar to probe B except that a very dark background was apparent throughout the tracks containing RNA from the human cell lines (data not shown). This is presumably a consequence of the repetitive element present in this clone. Probe A showed no hybridization to the three major RNA species on Northern blots and also failed to cross-hybridize with sequences in mouse DNA (Fig. 1B, lane k). Both these observations are consistent with the fragment being part of an intron; such a sequence would not be present in mRNA and is likely to have diverged considerably between mouse and man.

Two pieces of information strongly indicate that the 2,200 nucleotide RNA species is the major transcript from the human transforming gene: (1) it is the only transcript absent from NIH/3T3 cells (lane f) and present in transfectants (lanes d, e); and (2) it is known that *BglII*, *BamHI* and *SstI* (unpublished

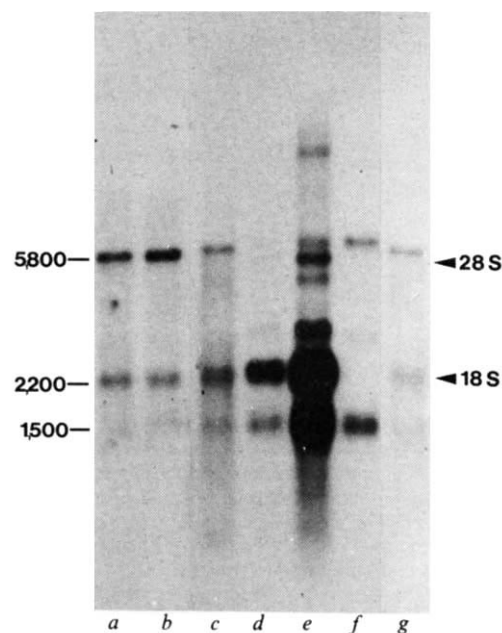


Fig. 2 Northern blots using probe B to identify the transforming gene transcript. Lanes are as follows: a, B6FS, a human fibrosarcoma cell line lacking a detectable transforming gene; b, normal human fibroblasts (FLOW 2002); c, HT1080; d, an HT1080 transfectant; e, an RD transfectant; f, NIH/3T3; g, EJ, a human bladder tumour line.

Methods: Cells were lysed in 10 mM Tris, pH 7.5, 0.1 M NaCl, 0.1 mM EDTA and 1% SDS, followed by sonication, phenol extraction and treatment with RNase-free DNase. Total RNA was passed once through an oligo(dT) cellulose column and poly(A) containing mRNA was eluted with water. mRNA (3 µg) samples were heated at 60 °C for 5 min in 50% de-ionized formamide, 2.2 M formaldehyde and 20 mM sodium phosphate pH 7.0 and then electrophoresed at 2 V cm⁻¹ overnight through an 0.8% agarose gel containing 20 mM sodium phosphate, pH 7.0 and 2.2 M formaldehyde. The gel was blotted to nitrocellulose as described by Thomas³³ and the blot was hybridized with 50% formamide, 3×SSC and 10% dextran sulphate containing 10⁶ c.p.m. ml⁻¹ of nick-translated probe B at 42 °C for 20 h followed by washing with 0.1×SSC at 60 °C.

data) all destroy the transforming activity of HT1080 DNA. However, we have already shown that *BglII* sites 1 and 3 (Figs 1A, C) lie outside the transforming gene and therefore only *BglII* site 2, which is within probe B, can be located in the gene. Similarly, the *SstI* site in the cloned fragment (Fig. 1A) and the *BamHI* site in the uncloned segment of DNA (Fig. 1C) must lie within the transforming gene. The 2,200 nucleotide mRNA detected by probe B must, therefore, be derived from the transforming gene.

Finally, the amount of the 2,200 nucleotide transcript in normal human fibroblasts (Fig. 2, lane b) is similar to that in HT1080 cells (lane c) or in RD cells (data not shown). It is therefore unlikely that enhanced gene expression is the mechanism of activation of this oncogene. We have also detected this transcript in a human fibrosarcoma cell line, B6FS (lane a), which does not have a detectable transforming gene (unpublished results) and in EJ (lane g) which contains a different transforming gene, c-Ha-ras1.

Homology with *ras* gene family

We have shown that the transforming sequence present in the two human sarcoma cell lines does not have detectable homology to 15 cloned viral oncogenes (ref. 4 and unpublished results). Recently, however, Chang *et al.*¹¹ have identified, by probing human gene libraries with v-Ha-as and v-Ki-ras probes, four distinct members of a human *ras* gene family and in the conditions we used to analyse Southern blots of transfectants we would have detected only two of these—c-Ha-ras1

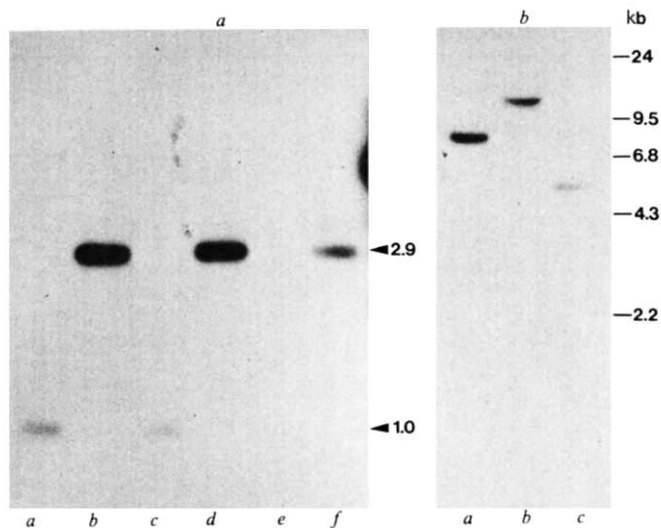


Fig. 3 **A**, Detection of homology with the cloned v-Ki-ras and the human c-Ha-ras1 genes. Lanes a, c, e, 0.2 μ g of *Eco*RI digested HiHi-3²⁰ (the v-Ki-ras oncogene is located on a 1.0 kb fragment); lanes b, d, f, 0.2 μ g of *Sst*I digested pHa-1¹¹ (the c-Ha-ras1 gene is located on a 2.9 kb *Sst*I fragment). The blots were hybridized in 50% formamide, 3 $\times$ SSC, 10% dextran sulphate and 10⁶ c.p.m. ml⁻¹ of nick-translated probe B at 42 °C for 20 h and washed as follows: lanes a and b, 0.6 $\times$ SSC, 50 °C; lanes c and d, 0.6 $\times$ SSC, 55 °C; lanes e and f, 0.6 $\times$ SSC, 60 °C. **B**, Analysis of the transforming gene present in HL60 and SK-N-SH cell lines. *Eco*RI digests of DNA derived from NIH/3T3 cell transformed by HT1080 (lane a), SK-N-SH (lane b) and HL60 (lane c) DNAs were electrophoresed through an 0.8% agarose gel and blotted onto nitrocellulose. The Southern blot was hybridized at 42 °C for 20 h with 50% formamide, 3 $\times$ SSC, 10% dextran sulphate and 10⁶ c.p.m. ml⁻¹ of nick-translated probe A and then washed in 0.1 $\times$ SSC at 60 °C.

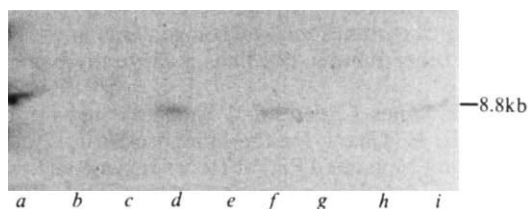


Fig. 4 Detection of the human N-ras gene in mouse: human hybrids. Southern blot of DNA from mouse: human hybrids hybridized with probe A. DNAs are as follows: lane a, human; b, mouse; c, CTP41P2; d, SIR19A; e, FIR5R3; f, CTP34B4; g, DUR4.3; h, 3W4C110; i, SIR7411. Hybridization conditions were as described in the legend to Fig. 3B.

and c-Ki-ras2. It was therefore important, now that a cloned segment of the sarcoma transforming gene had been obtained, to test again for homology with cloned *ras* probes. Figure 3A shows that in conditions of low stringency of washing, homology could be detected between probe B (Fig. 1A) and both v-Ki-ras (lanes a, c, e) and human c-Ha-ras1 (lanes b, d, f). After comparing the restriction maps of the sarcoma transforming gene with those published for the four known human *ras* genes¹¹ it is clear that the sarcoma transforming gene must be a novel member of the *ras* gene family, not previously identified. Furthermore, Southern blot analysis using various stringencies of washing reveals that the T_m for probe B: c-Ha-ras1 hybrids is about 60 °C at 0.09 M Na⁺, and we estimate that the sarcoma transforming gene and the c-Ha-ras1 gene have diverged at roughly 15% of their nucleotide sequences. (For comparison, it is known from sequence data^{18,19} that v-Ki-ras and v-Ha-ras differ at some 23% of their nucleotide protein-coding sequences.) Our failure to detect this transforming gene on Southern blots of DNA from HT1080 or RD transfectants using either v-Ha-ras or v-Ki-ras probes⁴ can be ascribed to this divergence in DNA sequence. It is interesting that in addition to the

transforming gene transcript two other species of RNA of 5,800 and 1,500 nucleotides are detected in both mouse and human cells (Fig. 2). These transcripts are almost identical in size to those observed by others using c-Ha-ras1 gene probes in human cells^{6,8} and v-Ki-ras probes²² in mouse cells. It is not clear which species are the authentic messages corresponding to the p21 proteins.

Recently a transforming gene has been cloned from HL60, a promyelocytic leukaemia line (J. M. Cunningham, M. J. Murray and R. A. Weinberg, personal communication) and from SK-N-SH, a human neuroblastoma line²³. On the basis of *Alu* blots¹⁻⁴ these genes were previously thought to be distinct from each other and from the transforming gene described here. However, after exchanging clones with Cunningham, Murray and Weinberg, we and they have shown that the restriction maps of part of their HL60 clone and of our HT1080 clone are identical. Furthermore, probe A (Fig. 1A), which is probably an intron sequence and which does not cross-hybridize with any other sequence in mouse or human DNA, hybridizes with DNA isolated from HT1080, SK-N-SH or HL60 transfectants (Fig. 3B, lanes a, b, c, respectively). We conclude that the same transforming gene is activated in the four cell lines HT1080, RD, HL60 and SK-N-SH and after consultation with Wigler and with Weinberg it has been proposed that this gene be called N-ras.

Chromosomal location of N-ras

To determine the chromosomal location of the N-ras gene we made use of a series of mouse:human hybrid cell lines (Table 1), containing different human chromosomes. DNA was isolated from each cell line, digested with *Eco*RI and analysed on Southern blots. Probe A (Fig. 1A) was used as this was known not to cross-hybridize with mouse sequences. Figure 4 shows the results obtained with DNA from some of the hybrid cell lines. Out of 17 hybrid cell lines analysed, 7 contained chromosome 1 and hybridized with the N-ras probe (Table 1). Ten hybrids did not contain chromosome 1, and none of these hybridized with the probe. It is clear, therefore, that N-ras is located on chromosome 1. This is in contrast to the other members of the *ras* family which are located on human chromosomes 11 (c-Ha-ras1)²⁴, X (c-Ha-ras2)²⁵, 6 (c-Ki-ras1)²⁵ and 12 (c-Ki-ras2)²⁵.

Discussion

We have isolated a molecular clone containing part of the transforming gene previously identified by us in two human sarcoma cell lines, and shown it to be a novel member of the *ras* gene family. Various unique sequence probes derived from this clone have been used to construct a partial restriction map of the whole of the transforming gene, and its surrounding sequences, in HT1080, RD and in DNA isolated from normal cells. From these restriction patterns it is clear that the gene is not grossly rearranged in the tumour cell lines, and furthermore it appears that gene amplification is not required for its transforming activity. We have identified the major transcript of the transforming gene as being a 2,200 nucleotide species that is present at similar levels in the two sarcoma cell lines, in normal fibroblasts and in various other tumours either lacking a detectable transforming gene or containing a different transforming gene. Enhanced levels of transcription from this gene do not appear to be responsible for its transforming activity. From these results we would tentatively expect that the mechanism of activation of this gene will be an alteration in the nucleotide sequence leading to a change in the amino acid sequence of the gene product. Since we have also been able to show that this is a new member of the *ras* gene family (now called N-ras) the mechanism of activation described above is also suggested by analogy with the known mechanism of activation of another *ras* gene, c-Ha-ras1, in the human bladder tumour line EJ^{13,14}. In the case of c-Ha-ras1 activation, a glycine residue at position 12 of the normal p21 protein has been altered to a valine

Table 1 Hybridization of an N-ras specific probe to DNA from mouse:human cell hybrids

Presence of 8.8 kb <i>Eco</i> RI fragment		Human chromosomes present in hybrid cell lines																						
Hybrid		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
CTP41p2	-			+			+	+							+		+	+			+			+
SIR19A	+	+	+	+		+		+					+		+	+			+	+		+		+
CTP34B4	+	+	+			+	+	+					tr	+	+		+	tr	+					+
DUR 4.3	-			+		+					+	+	+	+	+	+		+	+		+	+	+	+
3W4C110	-	tr									+	+	+		+			+	+		+	+	+	+
SIR7411	+	+	+	tr								+	+		+				tr			+	tr	+
C10b	-			+						+	+				+							+	+	
FIR5R3	-														+				+					
F4SC13C112	+	+								+					+									+
F4SC13C119	+	+													+									+
HORLI	-																+							
HORL9D2R1	-											+												
HORL9X	-																							+
Mog 13/9	-																							+
Mog 13/10	+	+	+												+							+	+	+
Mog 13/17	-																					+	+	+
Mog 13/22	+	+																				+	+	+

Most of the hybrids used in this study have been described by Heisterkamp *et al.*³⁴. Exceptions are the Mog hybrids³⁵, CTP34B4³⁶, 3W4C110³⁷ and the SIR hybrids³⁸. The human chromosome content of the hybrids was deduced by karyotypic^{39,40}, antigenic⁴¹ and enzymatic analyses⁴².

+ Indicates the presence of a particular human chromosome; tr, the presence of part of a chromosome as a translocation.

residue. Furthermore, the first 36 amino acid residues of v-Ha-ras¹⁸ and v-Ki-ras¹⁹ are identical to those of the human c-Ha-ras1 protein¹⁴ except that once again the amino acid at position 12 has been substituted—to arginine, in the case of v-Ha-ras, and to serine in v-Ki-ras. It remains to be seen whether the activation of N-ras in both HT1080 and in RD involves a similar alteration.

From these studies using transfection and those of others screening human libraries with v-Ki-ras and v-Ha-ras probes, five members of the human *ras* gene family have now been identified. c-Ha-ras1 and c-Ki-ras2 both encode p21 proteins²⁰, but it is not yet known whether the other members of the *ras* family also encode p21 proteins. The results of Northern blotting presented here and elsewhere⁶ clearly indicate that N-ras and c-Ha-ras1 are expressed not only in normal fibroblasts but in many tumour cells. It appears that both gene products are required for normal cell growth and disturbances to either may lead to malignant transformation. It is interesting that although other members of the *ras* gene family which have been activated in human tumours are also found incorporated into animal retroviruses^{12,16,26}, no transforming retrovirus containing N-ras has yet been described.

Recently there has been great interest in the role of chromosomal rearrangements in activating cellular oncogenes, particularly c-myc^{27–30} and c-abl³¹. We have shown that N-ras is located on chromosome 1. Alterations in chromosome 1, par-

ticularly trisomy, are frequently found in human tumours³²; indeed HT1080 itself has a translocation involving chromosome 1 (B. Reeves, personal communication). Our results comparing restriction maps of N-ras in normal and in HT1080 DNA, however, show no rearrangements within 1 kb either side of this gene. Nevertheless, it is possible that certain mechanisms of activation of cellular oncogenes, for instance chromosomal rearrangements, may have led to genetic changes in tumours that are not detectable by transfection into NIH/3T3 cells. The possible role of chromosomal rearrangements in the activation of N-ras in other tumour cell lines is currently being investigated.

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Note added in proof: We have recently cloned the sequence downstream of the 8.8 kb *Eco*RI fragment of HT1080. *In vitro* ligation of cloned sequences stretching from the *Bam*HI site in pAT8.8 through to the *Sst*I site in the previously uncloned segment (Fig. 1C) efficiently transform NIH/3T3 cells as predicted in this article.

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- Murray, M. J. *et al.* *Cell* **25**, 355–361 (1981).
- Perucho, M. *et al.* *Cell* **27**, 467–476 (1981).
- Lane, M. A., Saiten, A. & Cooper, G. M. *Cell* **28**, 873–880 (1982).
- Marshall, C. J., Hall, A. & Weiss, R. A. *Nature* **299**, 171–173 (1982).
- Pulciani, S. *et al.* *Nature* **300**, 539–542 (1982).
- Goldfarb, M., Shimizu, K., Peruchio, M. & Wigler, M. *Nature* **296**, 404–409 (1982).
- Shih, C. & Weinberg, R. A. *Cell* **29**, 161–169 (1982).
- Santos, E. *et al.* *Nature* **298**, 343–347 (1982).
- Parada, L. F., Tabin, C. J., Shih, C. & Weinberg, R. A. *Nature* **297**, 474–478 (1982).
- Der, C. J., Krontiris, T. E. & Cooper, G. M. *Proc. natn. Acad. Sci. U.S.A.* **79**, 3637–3640 (1982).
- Chang, E. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **79**, 4848–4852 (1982).
- Ellis, R. W. *et al.* *J. Virol.* **36**, 408–420 (1980).
- Tabin, C. J. *et al.* *Nature* **300**, 143–149 (1982).
- Reddy, E. P., Reynold, R. K., Santos, E. & Barbacid, M. *Nature* **300**, 149–152 (1982).
- Tapartowsky, E. *et al.* *Nature* **300**, 762–765 (1982).
- Kirsten, W. H. & Myer, L. A. *J. natn. Cancer Inst.* **39**, 311–335 (1967).
- Der, C. J. & Cooper, G. M. *Cell* **32**, 201–208 (1983).
- Dhar, R. *et al.* *Science* **217**, 934–937 (1982).
- Tsuchida, N., Ryder, T. & Ohtsubo, E. *Science* **217**, 937–939 (1982).
- Ellis, R. W. *et al.* *Nature* **292**, 506–511 (1981).
- Twigg, A. J. & Sherratt, D. *Nature* **283**, 216–218 (1980).
- Ellis, R. W., Defeo, D., Furth, M. E. & Scolnick, E. M. *Molec. Cell Biol.* **2**, 1339–1345 (1982).
- Shimizu, K., Goldfarb, M., Peruchio, M. & Wigler, M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 383–387 (1983).
- McBride, O. W. *et al.* *Nature* **300**, 773–774 (1982).
- O'Brien, S. J., Nash, W. G., Goodwin, G. L., Lowy, D. R. & Chang, E. *Nature* **302**, 839–842 (1983).
- Anderson, P. R. *et al.* *Cell* **26**, 129–134 (1981).
- Taub, R. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **79**, 7837–7841 (1982).
- Dalla-Favera, R. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **79**, 7824–7827 (1982).
- Crews, S. *et al.* *Science* **218**, 1319–1321 (1982).
- Sheng-Ong, G. L. C., Keath, E. J., Piccoli, S. P. & Cole, M. D. *Cell* **31**, 443–452 (1982).
- DeKlein, A. *et al.* *Nature* **300**, 765–767 (1982).
- Rowley, J. D. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5729–5733 (1977).
- Thomas, P. S. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5201–5205 (1980).
- Heisterkamp, N. *et al.* *Nature* **299**, 747–749 (1982).
- Povey, S. *et al.* *Ann. hum. Genet.* **43**, 241–248 (1980).
- Jones, E. A. *et al.* *Somatic Cell Genet.* **2**, 483–496 (1976).
- Nabholz, M., Miggiano, V. & Bodmer, W. F. *Nature* **223**, 358–363 (1969).
- Whitehead, A. S. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **79**, 5021–5025 (1982).
- Bobrow, M. & Cross, J. *Nature* **251**, 74–79 (1974).
- Casperson, T., Lomakka, G. & Zech, L. *Hereditas* **67**, 89–102 (1971).
- Goodfellow, P. N. & Solomon, E. in *Monoclonal Antibodies in Clinical Medicine* (eds McMichael, A. & Folvie, J.) 365–393 (Academic, London, 1982).
- Harris, H. & Hopkinson, D. A. *Handbook of Enzyme Electrophoresis in Human Genetics* (North-Holland, Amsterdam, 1976).

Translocation, breakage and truncated transcripts of *c-myc* oncogene in murine plasmacytomas

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Comparative nucleotide sequence analysis of a rearranged c-myc gene in a murine plasmacytoma and c-myc cDNA from normal spleen reveals that chromosomal translocation in the plasmacytoma breaks the c-myc gene within the first exon or intron. In the plasmacytoma truncated c-myc RNAs initiate from newly exposed promoter sites. Nevertheless, the myc polypeptide produced in the plasmacytoma is probably the same as that from the intact c-myc gene because the exon lost by breakage and translocation is non-coding. The second and third exons of the mouse c-myc gene are substantially conserved in the v-myc gene of the avian retrovirus, MC29.

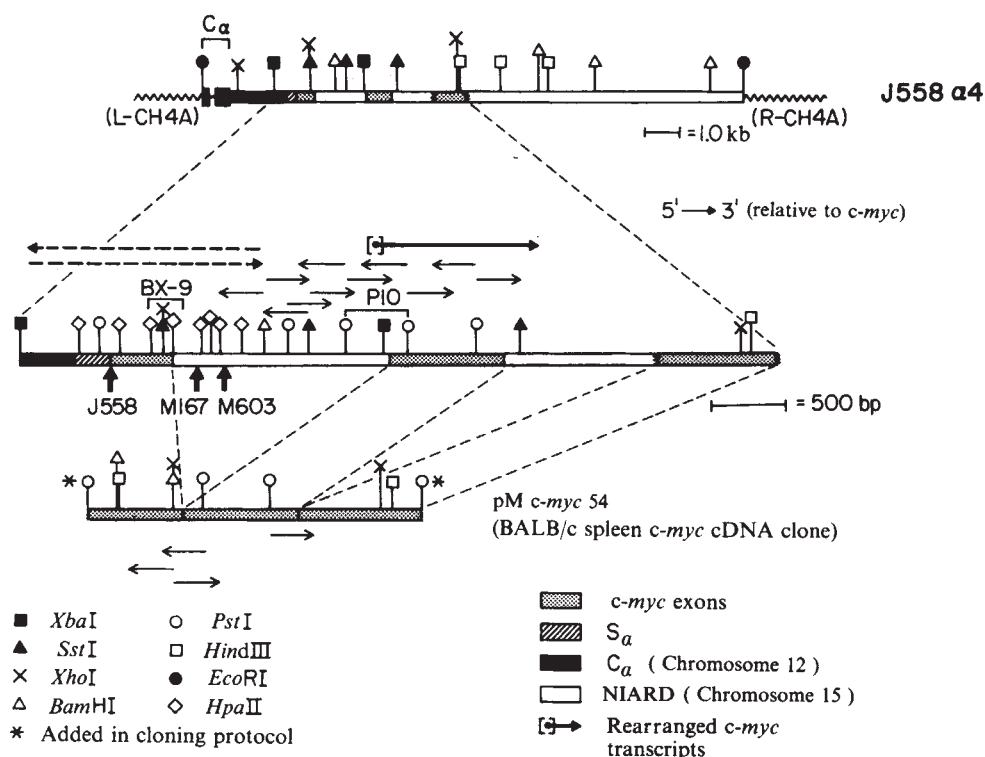
A VARIETY of lymphoproliferative cancers of mice and men are known to possess characteristic and unique chromosome translocations^{1,2}. Until recently, the potential significance of specific chromosome abnormalities for cellular neoplastic transformation has remained obscure. Molecular studies have now demonstrated that various cellular homologues of retroviral oncogenes are associated with specific chromosome translocations in murine plasmacytomas (*c-myc*)³⁻⁹, Burkitt lymphomas (*c-myc*)^{7,9-11}, acute non-lymphoblastic leukaemias (*c-mos*)¹⁰ and chronic myelocytic leukaemia (*c-abl*)¹² of man.

We have previously shown that NIARD (for non-immunoglobulin-associated rearranging DNA)¹³, a DNA sequence associated with T(12; 15) (or rcpT(12; 15)) chromosome translocations in murine plasmacytomas³, contains the *c-myc* oncogene⁹. NIARD (that is, *c-myc*) rearrangements are observed in most mouse plasmacytomas^{4-6,13,14} but not in normal lymphocytes^{4,5,13}. The human *c-myc* gene translocates with the distal end of chromosome 8 to the immunoglobulin heavy-chain

gene locus on chromosome 14 in several Burkitt lymphomas^{7,10}, where it may reside adjacent to the μ heavy-chain constant region gene^{7,9}. Presumably, an analogous translocation of the mouse *c-myc* oncogene from chromosome 15 to the immunoglobulin heavy-chain gene locus on chromosome 12^{3,4,7} (or the reciprocal translocation, rcpT(12; 15))³ contributes to the production of novel *c-myc* transcripts which are ~400 nucleotides smaller and 10–20 times more abundant than the *c-myc* RNAs detected in normal mouse cells^{5,6,9,15}. BALB/c IgA-producing plasmacytomas translocate NIARD to the C_{α} gene switch region (S_{α}), thereby generating an aberrantly rearranged C_{α} gene^{3-6,13,14}. Some non-IgA-producing BALB/c plasmacytomas^{4,5,13} and most NZB mouse plasmacytomas¹³ rearrange NIARD independently of S_{α} . However, comparable levels of rearranged *c-myc* transcripts are observed irrespective of the NIARD translocation site^{5,6,9,15}.

We show here that NIARD translocation in murine plasmacytomas breaks the *c-myc* oncogene within the 5'-most of three

Fig. 1 Location of mouse *c-myc* exons within the cloned J558 myeloma NIARD rearrangement (J558 α 4)^{9,13} and a mouse *c-myc* cDNA clone. A 2.2-kb cDNA clone (pM *c-myc* 54) of the mouse *c-myc* mRNA was isolated from a BALB/c spleen cDNA library by using a human *c-myc* cDNA probe (pRyc-7.4)⁹ (R.W., unpublished results). The cDNA was cloned into the *Pst*I site of pBR322 by poly(dC-dG) tailing. Thin and broken overhead arrows indicate DNA sequencing strategies. The nucleotide sequence of the region denoted by two long broken arrows was determined from numerous overlapping *Hae*III and *Hpa*II restriction fragments. The precise 5' and 3' boundaries of the third *c-myc* exon in J558 α 4 remain to be determined and are indicated by vertical saw-toothed lines.



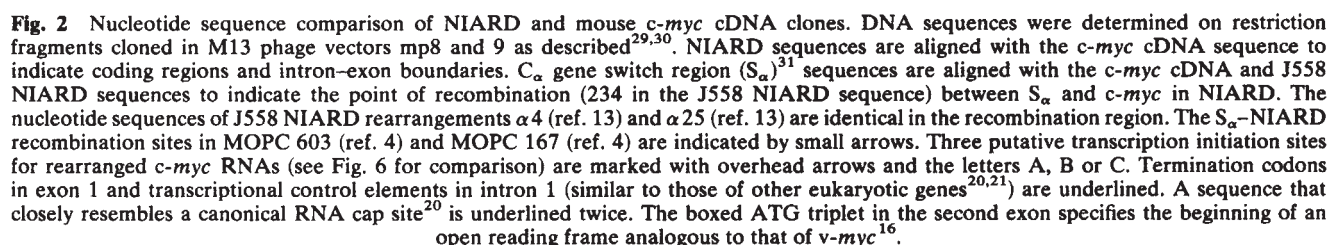
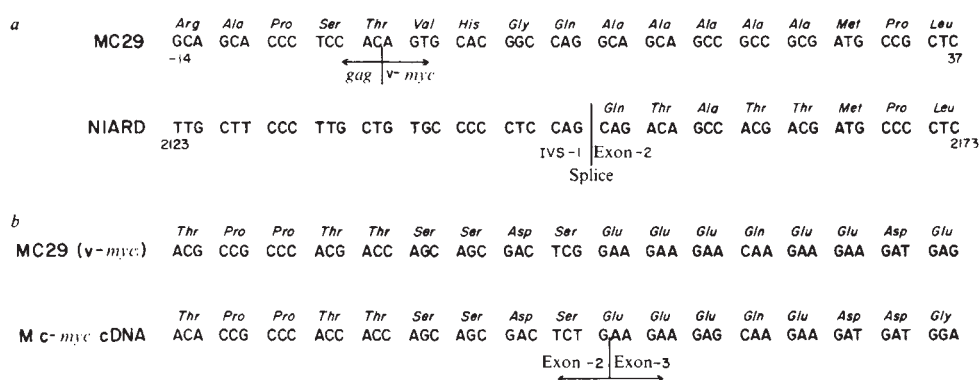


Fig. 3 MC29 *v-myc*¹⁶ and mouse *c-myc* sequence comparisons at exon boundaries. *a*, Nucleotide sequences of MC29 viral genome at the *gag-myc* boundary¹⁶ is compared with the J558 NIARD sequence at the intron 1/exon 2 boundary. Numbers refer to nucleotide positions in MC29¹⁶ and J558 NIARD (see Fig. 2). *b*, Comparison of nucleotides 690–740 of the MC29 *v-myc* gene¹⁶ to our mouse *c-myc* cDNA sequence at the exon 2/exon 3 boundary.



c-myc exons or within the first *c-myc* intron. The altered context of the broken *c-myc* gene then contributes to the production of rearranged *c-myc* transcripts which may initiate from multiple sites residing within the first *c-myc* intron. The rearranged *c-myc* gene displays extensive homology with the *v-myc* gene of MC29.

NIARD-associated chromosome translocation breaks *c-myc* oncogene

To determine precisely the boundaries of the rearranged *c-myc* gene encoded within a translocated NIARD sequence, we determined the nucleotide sequence of ~2.9 kilobases (kb) of the J558 NIARD rearrangement starting from the *S_a* recombination site. We then compared this sequence with that obtained from a 2.2-kb *c-myc* cDNA clone isolated from a normal mouse spleen cDNA library. As shown in Figs 1 and 2, the *S_a* region has aberrantly recombined with the first exon of the mouse *c-myc* gene in the J558 myeloma. Therefore, this recombination event breaks the 5'-most *c-myc* exon, resulting in the removal of the normal *c-myc* promoter region. The NIARD rearrangement site in J558 possesses one additional T residue which is absent from both *S_a* and *c-myc*⁵ (see Fig. 2). Two distinct NIARD rearrangements were previously cloned from J558 DNA ($\alpha 4$ and $\alpha 25$)¹³. The *S_a* and NIARD recombination sites of $\alpha 25$ are identical to those displayed for $\alpha 4$ in Fig. 2, with both possessing an extra T at the site of recombination. These results strongly imply that one of the two J558 NIARD rearrangements¹³ is the precursor of the other. This conclusion is in keeping with our previous observation that all NIARD rearrangements 3' of *c-myc* also contain a rearrangement 5' of *c-myc*¹³. The NIARD rearrangement present in MPC-11 cells was previously shown to occur outside the *C_H* gene locus¹³. Recent data now indicate that the MPC-11 site resides on chromosome 12 and as in the J558 case breaks the first *c-myc* exon (L. J. Harris, L.W.S. and K.B.M., unpublished data). The NIARD-*S_a* rearrangements found in M167 and M603⁴ occur 674 and 792 base pairs (bp), respectively, downstream from the J558 sites within NIARD and therefore reside within the first *c-myc* intron (see Figs 1, 2).

The ATG triplet at position 2,166 of the NIARD sequence (position 16 of *c-myc* exon 2) defines an open reading frame and could initiate a polypeptide encoded by the truncated *c-myc* RNA. In addition, termination codons are present in all three reading frames within the first *c-myc* exon which is broken by chromosome translocation (see Fig. 2). Therefore, a normal-sized *c-myc* transcript may not encode a larger protein than that specified by a truncated *c-myc* RNA. More direct studies on the nature of the proteins encoded by normal and rearranged *c-myc* RNAs will help to confirm this observation.

The 5' and 3' boundaries of the third *c-myc* exon remain tentative. The size of the second intron has been estimated by restriction mapping and R-loop analysis^{6,9}. Northern blotting of nuclear RNAs confirms the existence of an RNA precursor which is ~1 kb larger than the mature rearranged RNAs, in agreement with the presence of a second intron of ~1 kb (R. P. Perry and K.B.M., unpublished results). Assuming our *c-myc*

cDNA clone is close to full length (~2.2 kb compared with an estimated *c-myc* RNA size of ~2.3 kb), the presence of additional *c-myc* exons beyond that denoted in Fig. 1 may be unlikely. However, complete sequence analysis of the mouse *c-myc* cDNA and genomic clones will be required to confirm whether other 5' and 3' *c-myc* exons exist.

Comparative anatomy of avian-derived MC29 *v-myc* and murine *c-myc* genes

A comparison of the *c-myc* sequence presented here and the MC29 *v-myc* sequence¹⁶ gives some insights into the mechanism for transduction of avian *c-myc* sequences into the genome of the avian myelocytomatosis virus, MC29¹⁷. The conservation of reading frames between the *v-myc* gene of MC29 and the mouse *c-myc* gene is consistent with the proposed model of cellular oncogene capture by retroviruses¹⁷. Figure 3*a* compares the nucleotide sequence at the boundary between intron 1 and exon 2 of *c-myc* with the sequence of the MC29 genome near the *gag-myc* fusion boundary¹⁶. The best match of coding sequences predicts that the fusion of *gag* to *myc* occurred within the first intron 13 nucleotides 5' to the beginning of exon 2 of the *c-myc* gene. This would agree with the report that the *c-myc*-transduced cellular domains have leftward boundaries within introns¹⁸. As a result of the *gag-myc* fusion, a small portion of the *c-myc* intron provides amino acid coding information to bridge the gap between *gag* and the second *c-myc* exon of the *gag-myc* polypeptide. A search of *v-myc* for a region corresponding to the exon 2/exon 3 boundary of the mouse *c-myc* gene reveals conservation of reading frame continuity (see Fig. 3*b*). This region shows 86% nucleotide sequence homology, with most differences residing at the third position of the codons, resulting in essentially complete amino acid conservation.

A gross comparison of *v-myc* to the mouse and human *c-myc* sequences (L.W.S., R.W. and K.B.M., unpublished results) reveals two highly conserved regions. The first 230 nucleotides of *v-myc* are closely homologous (~70%) to the corresponding region within exon 2 of mammalian *c-myc*. A more extensive region of homology exists between *v-myc* and *c-myc* at the end of exon 2 and the entire third exon encompassing ~600 nucleotides. This region codes for many basic amino acids¹⁶ and has been suggested to facilitate the DNA binding capability of the *v-myc* gene product¹⁹. Further speculation on the importance of this region for oncogenic transformation must await a more rigorous comparison of avian *v-myc* and mammalian *c-myc* genes (L.W.S., R.W. and K.B.M., in preparation).

NIARD rearrangements may represent reciprocal translocations

We next determined the fate of the 5' end of the *c-myc* gene which is lost as a consequence of NIARD translocation. As shown in Fig. 4, Southern blots of restricted BALB/c, J558 and M167 DNAs were hybridized to a *Pst*I fragment obtained from the *c-myc* cDNA clone (pM *c-myc* 54 in Fig. 1) which contains the first exon and part of the second exon. Unexpectedly, three major homologous sequences are detected in

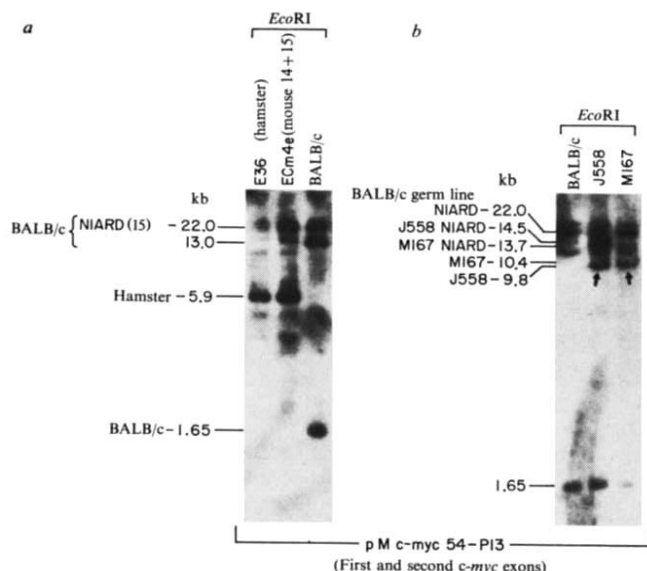


Fig. 4 Southern hybridizations performed with a combined *c-myc* cDNA exon 1 and 2 probe and various restricted DNA samples. The 5'-most *Pst*I fragment of the *c-myc* 54 cDNA clone (see Fig. 1) was subcloned into M13 mp8 (denoted pM *c-myc* 54-p13) and labelled as described previously³². 7.5 µg of each DNA sample were digested with *Eco*RI and applied to a 1% agarose gel, transferred to nitrocellulose³³ and hybridized as described previously^{9,34}. Bands indicated by arrows only hybridize to the 5'-most *Pst*I fragment of pM *c-myc* 54.

BALB/c DNA (see Fig. 4). One of the sequences corresponds to the anticipated chromosome 15 associated NIARD sequence but the other two were not previously observed with a probe specific for the second and third *c-myc* exons. These *c-myc*-related sequences are homologous to the first exon and do not reside on either mouse chromosomes 14 or 15 (see Fig. 4a). The origin and nature of these sequences will not be examined further here. In addition to these *c-myc*-positive bands, J558 and M167 DNAs each contain two rearranged NIARD sequences: (1) a NIARD sequence containing the second and third exons of *c-myc*⁹ (Figs 1, 2); (2) a NIARD sequence specific for the first exon of *c-myc*. We will refer to these sequences as 3' NIARD and 5' NIARD, respectively. 5' NIARD could meet the criteria for the product of a reciprocal translocation (see cytogenetic model b in ref. 3). The NIARD rearrangement sites in J558 and M167 are 674 bp apart. Thus, the minor differences in the sizes of the 5' NIARD fragments are essentially in keeping with the sizes of their 3' NIARD counterparts. Molecular cloning of these 5' NIARD fragments will help to confirm the notion of a reciprocal NIARD translocation.

Rearranged *c-myc* transcripts initiate within the first *c-myc* intron

As shown in Fig. 5, we have extended our previous Northern blotting experiments on poly(A)⁺ RNAs isolated from normal mouse spleen and plasmacytomas with (+) and without (−) NIARD rearrangements⁹ by using DNA probes specific for *c-myc* exons and introns. These results indicate that: (1) only normal mouse spleen *c-myc* transcripts and the *c-myc* transcripts of a plasmacytoma with no apparent NIARD rearrangement (that is, PC3741)¹³ hybridize to the 5'-most *c-myc* exon⁵ (see Fig. 5a); (2) *c-myc* transcripts present in plasmacytomas harbouring NIARD rearrangements (J558, MPC-11 and H2020 in ref. 9) hybridize uniquely to the first *c-myc* intron (see Fig. 5b); (3) both normal and rearranged *c-myc* RNAs hybridize to the second *c-myc* exon^{5,9} (see Fig. 5c). The rearranged *c-myc* RNAs are easily detected with a 402-bp *Pst*I fragment (1,885–2,287 of the NIARD sequence in Fig. 2) which

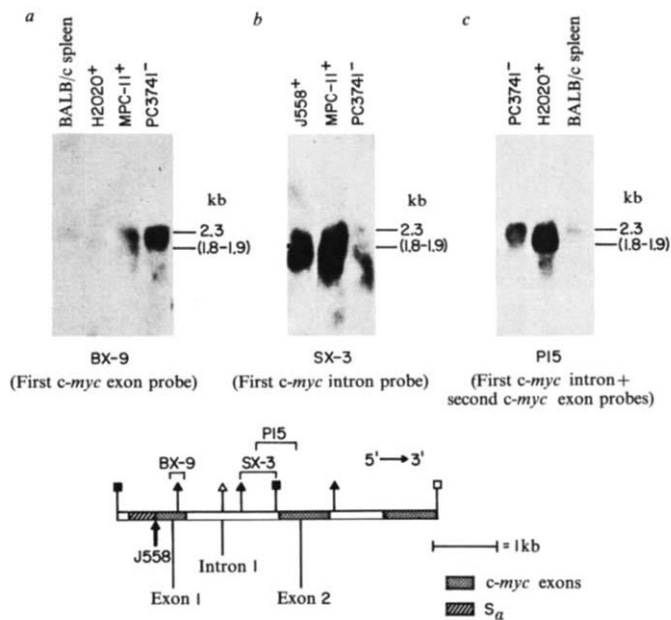


Fig. 5 Northern hybridizations performed with various poly(A)⁺ RNAs and *c-myc* exon and intron probes. Plus and minus signs indicate the presence or absence of NIARD rearrangements. DNA probes were cloned in M13 mp8 phage. Northern transfers and hybridizations were performed as described elsewhere^{9,34,35}.

contains 265 bp of the first intron and 137 bp of the second exon (P15 in Fig. 5c). As rearranged *c-myc* RNAs did not hybridize to intron 1 sequences 5' of P15⁹ (data not shown), we reasoned that their initiation sites should lie within P15 between nucleotides 1,885 and 2,150 of the NIARD sequence (see Fig. 2).

To position the transcription initiation sites of the rearranged *c-myc* RNAs precisely within the first *c-myc* intron, we next performed S₁ nuclease mapping with a ³²P-labelled P15 sequence as shown in Fig. 6. A novel procedure was used to prepare an internally labelled, single-stranded DNA probe (see Fig. 6 legend and L.W.S. and K.B.M., unpublished results). As shown in Fig. 6, three S₁ nuclease-resistant bands are observed when P15 is hybridized to poly(A)⁺ RNA from plasmacytomas with NIARD rearrangements. Poly(A)⁺ RNA from a plasmacytoma showing no NIARD rearrangement (PC3741)¹³ and total yeast RNA do not protect P15 sequences from S₁ digestion. Similar mapping studies performed with the *Pst*I fragment immediately 5' to P15 yielded no S₁-resistant bands with rearranged *c-myc* RNAs, in agreement with our Northern blotting results (data not shown). Therefore, J558, H2020 and MPC-11 transcripts each seem to initiate at one major and two minor sites within the first *c-myc* intron (that is, 166, 129 and 78 bp 5' of the second exon present in the normal mouse spleen *c-myc* cDNA clone). Promoter-like sequences common to eukaryotic genes are only found 5' of the first start site. All three putative initiation sites fall within or near a stretch of 20 CAs present in the first *c-myc* intron. The alternative mapping of transcription start sites by DNA primer extension will help to confirm these observations. The existence of multiple initiation sites in this region is somewhat in keeping with the disperse size range (1.8–2.0 kb) of the rearranged *c-myc* transcripts detected by Northern blotting^{5,6,9} (see Fig. 5). However, it is possible that either differential splicing of 3' exons (residing beyond the last *c-myc* exon shown in Fig. 1) and/or the existence of alternative poly(A) addition sites contributes to the relatively disperse sizes of the rearranged *c-myc* RNAs. Further studies designed to pinpoint the *c-myc* poly(A) addition site should settle this issue.

Conclusions

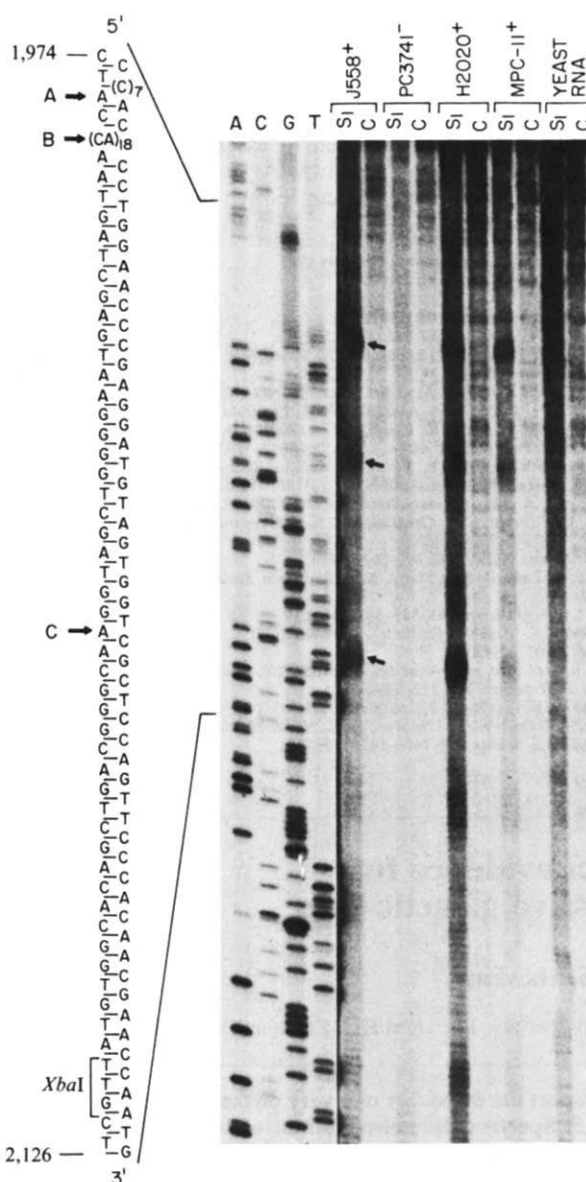
We have previously shown that the *c-myc* oncogene is contained within NIARD, a DNA sequence associated with the T(12; 15) chromosome translocation commonly observed in murine plasmacytomas^{3,9,13}. NIARD exists in a rearranged context in ~90% of all mouse plasmacytomas thus far tested (refs 4–6, 13, 14 and K.B.M. and L. J. Harris, unpublished results).

We have now conclusively demonstrated that *c-myc* is physically broken by a NIARD rearrangement within either the first *c-myc* exon (J558) or intron (M167, M603). The altered context of the now interrupted *c-myc* gene then contributes to the activation of several putative transcription initiation sites in the first *c-myc* intron, within 166 bp 5' of the second *c-myc* exon. The first and major initiation sequence (CCCAACCAC) closely resembles a canonical RNA cap site (5'YYCAYYYYY3')²⁰ and a variant, Goldberg-Hogness TATA promoter sequence (GATAGTA)²⁰ resides 33 nucleotides 5' of this initiation site. In addition, the sequence GGCATTCT (which conforms to another putative control sequence, GGTGAATCT, commonly found ~70–80 bp 5' of most eukaryotic genes)²¹ lies ~115 bp 5' of the major initiation site (that is, site A in Fig. 2). The second and third initiation sites (B and C in Fig. 2) do not reside near such potential control elements. Conceivably, S₁ mapped sites B and C may represent the termini of 5' processed RNAs or possibly result from S₁ nuclease over digestion²² and need to be confirmed by other means.

Fig. 6 S₁ nuclease mapping of transcription initiation sites of rearranged *c-myc* RNAs within *c-myc* intron 1. An internally labelled, single-stranded DNA sequence identical to the NIARD *Pst*I fragment of mp8 clone P15 which encompasses the end of intron 1 and the beginning of exon 2 (see Fig. 5 for location of P15 within NIARD) was synthesized from its mp8 cloned complementary sequence, P10. A 25- μ l reaction mixture containing 0.5 μ g of mp8 P10 template, 10 ng of M13 pentadecamer sequencing primer, 7 mM Tris-HCl pH 7.5, 7 mM MgCl₂, 50 mM NaCl and 20 mM dithiothreitol was preincubated at 60 °C for 15 min. The reaction volume was increased to 50 μ l supplemented with 80 μ Ci of [α -³²P]dCTP ($\geq 3,000$ Ci mmol⁻¹), 50 μ M dATP, dGTP and dTTP, 2.5 μ M dCTP, 15 U of Klenow DNA polymerase and the incubation continued at 37 °C for 60 min after which time the level of dCTP was increased to 40 μ M for a final 10 min at 37 °C. The labelled DNA was purified by Sephadex G-100 chromatography, ethanol-precipitated, resuspended, digested with *Eco*RI, heat-denatured and the labelled single-stranded P10 complement (that is, P15) purified on a 7 M urea-6% polyacrylamide gel. The *Eco*RI digestion step completely removes mp8 vector sequences. The purified fragment is a ³²P-labelled single-stranded molecule which contains the entire 402-bp *Pst*I insert of mp8 P15. This fragment also contains vector sequences of 20 bp 5' and 40 bp 3' of the 402-bp insert. The P15 sequence is complementary to the *c-myc* RNA. Samples of the isolated single-stranded NIARD-P15 sequence were hybridized to poly(A)⁺ RNAs from J558, PC3741, H2020, MPC-11 and total yeast RNA. A reaction containing 2 μ g of poly(A)⁺ RNA, 1.5 $\times 10^7$ c.p.m. of [α -³²P]-labelled P15, 80% deionized formamide, 400 mM NaCl, 10 mM PIPES pH 6.4, 5 mM EDTA was preincubated at 70 °C for 10 min and then at 46 °C for 6 h³⁶. A portion of the reaction was diluted 10-fold in 0.3 M NaCl, 30 mM NaAc pH 4.8 and 3 mM ZnSO₄·7H₂O and digested with 70 U of S₁ nuclease³⁷. Samples with and without S₁ nuclease treatment were applied to a DNA sequencing gel adjacent to a sequencing ladder of mp8 P10. Predominant S₁ nuclease-resistant bands of identical sizes are uniquely found with poly(A)⁺ RNAs from J558, MPC-11 and H2020 which all possess NIARD rearrangements¹³ and produce rearranged *c-myc* RNAs⁹. PC3741 (which lacks a NIARD rearrangement¹³ and produces normal sized *c-myc* RNA⁹) displays a virtually identical pattern to that of yeast RNA control. The putative transcription initiation sites within the NIARD-P15 sequence are indicated by arrows and the letters A, B or C. The sizes of the protected fragments were adjusted upward by 40 bp to account for primer and vector sequences present 3' of the P15 insert in sequencing conditions but lost from the labelled P15 probe subsequent to S₁ digestion. Start site B is located within the (CA)₂₀ block at nucleotide 2,021 of the NIARD sequence (see Fig. 2). Minor, nonspecific background bands present in lanes with and without S₁ digestion are due to the lability of the high specific activity single-stranded probe.

A sequence composed of 20 contiguous CA dinucleotides is found in the vicinity of the new initiation sites. This repeating unit of alternating purines and pyrimidines has been shown to be an evolutionarily conserved moderately repetitive sequence^{23,24}. In addition, such a sequence has been implicated as a possible expression control element by virtue of its potential to undergo a transition to the Z-DNA conformation^{25–27}. It is conceivable that this (CA)₂₀ region in its translocated context may have an enhancing effect on *c-myc* expression. It is also possible that S_a sequences provide some enhancing effect for *c-myc* RNA production. However, *c-myc* gene rearrangements need not involve S_a or any other switch region, even though similar rearranged *c-myc* RNAs are produced^{5,6,9,13}. Further experiments are required to assess the significance of the (CA)₂₀ region and NIARD target sites for rearranged *c-myc* RNA production.

The molecular basis for NIARD rearrangements remains obscure. DNA sequences found at the J558, M167 and M603 recombination sites share no obvious homology (see Fig. 2). The absence of S_a-like repetitive sequences within NIARD rule out a recombination event entirely mediated by S region homology. The retention of a 5' *c-myc* exon in a rearranged context in both J558 and M167 could meet the criteria for the product of a reciprocal translocation event and merits further study. NIARD rearrangements may be selected for by *c-myc* gene breakage coupled to the activation of novel rearranged



c-myc RNAs. Plasmacytomas without truncated myc RNAs produce high levels of normal-sized myc RNAs.^{9,15} In addition, low levels of normal c-myc RNAs are produced from the normal c-myc gene in rearrangement positive tumours^{9,15} (see Fig. 5, A). These observations imply that translocations may occur far from c-myc and result in increased c-myc expression in some plasmacytomas and warrant further study. Further speculation will await the elucidation of a function for the c-myc gene product.

Genetic rearrangements may be a necessary step for the activation of a variety of cellular oncogenes. As described here, the breakage of the c-myc gene by chromosome translocation contributes directly to the production of truncated c-myc RNAs which are presumably associated with the oncogenic potential of c-myc. It is interesting that the transduction of the avian c-myc gene in the avian myelocytomatosis virus, MC29, also results in the loss of the first c-myc exon¹⁶. A comparison of the nucleotide sequences of rearranged murine and avian retroviral myc genes reveals a conserved reading frame starting with an ATG near the 5' end of exon 2. Since termination codons reside in all three reading frames in murine exon 1, the ATG in exon 2 could initiate the synthesis of identical polypeptides from normal and rearranged c-myc RNAs. Therefore, exon 1 would then be a 5' non-coding sequence of normal c-myc transcripts. Chromosome translocation would replace this long 5' non-coding sequence for a smaller one by activating new transcription initiation sites within intron 1. A smaller 5' leader sequence may increase the translational capacity of the c-myc gene. The second and third exons of both avian and murine c-myc genes may be sufficient for c-myc oncogenicity. More refined studies on human c-myc gene rearrangements in Burkitt lymphomas which involve the C_μ immunoglobulin gene^{7,9} will be required to assess their relationship to mouse c-myc rearrangements. At present, there is no evidence for rearranged human c-myc RNAs in Burkitt lymphomas²⁸.

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- Klein, G. *Nature* **294**, 313–318 (1981).
- Rowley, J. P. *Science* **216**, 749–751 (1982).
- Harris, L. J., D'Eustachio, P., Ruddle, F. H. & Marcu, K. B. *Proc. natn. Acad. Sci. U.S.A.* **79**, 6622–6626 (1982).
- Calame, K. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 6994–6998 (1982).
- Adams, J. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 6966–6970 (1982).
- Shen-Ong, G. L. C., Keath, E., Piccoli, S. P. & Cole, M. D. *Cell* **31**, 443–452 (1982).
- Taub, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 7837–7841 (1982).
- Crews, S., Barth, R., Hood, L., Prehn, J. & Calame, K. *Science* **218**, 1319–1321 (1982).
- Marcu, K. B. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 519–523 (1983).
- Dalla-Favera, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 7824–7827 (1982).
- Neel, B. G., Jhanwar, S. C., Chaganti, R. S. K. & Hayward, W. S. *Proc. natn. Acad. Sci. U.S.A.* **79**, 7842–7846 (1982).
- deKlein, A. *et al. Nature* **300**, 765–767 (1982).
- Harris, L. J., Lang, R. B. & Marcu, K. B. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4175–4179 (1982).
- Kirsch, I. R. *et al. Nature* **293**, 585–587 (1981).
- Mushinski, J. F., Bauer, S. R., Potter, M. & Reddy, E. P. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1073–1077 (1983).
- Alitalo, K. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 100–104 (1983).
- Varmus, H. E. *Science* **216**, 812–820 (1982).
- Klempnauer, K. H., Gonda, T. J. & Bishop, J. M. *Cell* **31**, 453–463 (1982).
- Donner, P., Greiser-Wilke, I. & Moelling, K. *Nature* **296**, 262–266 (1982).
- Corden, J. *et al. Science* **209**, 1406–1414 (1980).

Since submission of this article, a canonical poly(A) addition sequence (AAUAAA) has been found within 30 bp of an oligo (dT) stretch at the 3' end of the mouse c-myc cDNA clone (L.W.S., R.W. and K.B.M., unpublished results). This poly(A) addition signal would be located 3' of the HindIII site which defines the 3' end of the third c-myc exon in the J558 genomic clone as shown in Fig. 1. It remains to be demonstrated whether this is the sole poly(A) addition signal available to the rearranged c-myc RNAs. Furthermore Jerry Adams, Suzanne Cory and their colleagues (personal communication) conclude from RNA blotting data that a 5' c-myc exon is broken in the translocated J558 myc gene³⁸ and that the c-myc translocation is a reciprocal exchange^{38,39}.

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Note added in proof: An analogous 5' non-coding exon has been observed in a human c-myc cDNA clone⁴⁰. The human c-myc genomic sequence reported by Colby *et al.*⁴¹ lacks such a 5' non-coding exon and presumably starts within the first c-myc intron. In agreement with this conclusion, two TATA-like sequences, found ~700 bp 5' of the ATG in the first human c-myc coding exon⁴¹, are present in a homologous context within the first murine c-myc intron. These conserved TATA-like sequences may be important for the activation of translocated c-myc genes (M. Cole, personal communication). The 5' non-coding c-myc exon sequence reported here is ~2% in disagreement with an independently determined sequence for the J558 myc recombination site⁵ (S. Cory, personal communication). Nevertheless, multiple translation termination codons are retained in all reading frames of this exon.

- Benoist, C., O'Hare, K., Breathnach, R. & Chambon, P. *Nucleic Acids Res.* **8**, 127–142 (1980).
- Miller, K. G. & Sollner-Webb, B. *Cell* **27**, 165–174 (1981).
- Miesfeld, R., Krystal, M. & Arnheim, N. *Nucleic Acids Res.* **9**, 5931–5907 (1980).
- Hamada, H., Petrino, M. G. & Kakunaga, T. *Proc. natn. Acad. Sci. U.S.A.* **79**, 6465–6469 (1982).
- Vorlickova, M., Kypr, J., Stokrova, S. & Sponar, J. *Nucleic Acids Res.* **10**, 1071–1080 (1982).
- Zimmer, C., Tyman, S., Marck, C. & Guschlbauer, W. *Nucleic Acids Res.* **10**, 1081–1091 (1982).
- Singleton, C. K., Klysik, J. & Wells, R. D. (in preparation).
- Westin, E. H. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 2490–2494 (1982).
- Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463–5467 (1977).
- Messing, J., Crea, R. & Seeburg, P. H. *Nucleic Acids Res.* **9**, 309–322 (1981).
- Davis, M. M., Kim, S. K. & Hood, L. *Science* **209**, 1360–1365 (1980).
- Hu, N. T. & Messing, J. *Gene* **17**, 271–277 (1982).
- Southern, E. M. *J. molec. Biol.* **98**, 503–517 (1975).
- Wahl, G. M., Stern, M. & Stark, G. R. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3683–3687 (1979).
- Thomas, P. S. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5201–5205 (1980).
- Casey, J. & Davidson, N. *Nucleic Acids Res.* **4**, 1539–1552 (1977).
- Vogt, V. M. *Eur. J. Biochem.* **33**, 192–200 (1973).
- Adams, J., Gerondakis, S., Webb, E., Corcoran, L. M. & Cory, S. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1982–1986 (1983).
- Cory, S., Gerondakis, S. & Adams, J. H. *Embo J.* (in the press).
- Watt, R. *et al. Nature* (in the press).
- Colby, W. W., Chen, E. Y., Smith, D. H. & Levinson, A. D. *Nature* **301**, 722–725 (1983).

LETTERS TO NATURE

Direct evidence for a massive galactic halo

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Here I report the discovery of a very distant galactic RR Lyrae star, R15. Spectroscopic observations of the object show that it has a high negative radial velocity, implying a lower limit to the mass of the galaxy of $1.4 \times 10^{12} M_{\odot}$.

Preliminary results¹ from a programme to detect faint variables using COSMOS measures of UK 1.2-m Schmidt plates included several RR Lyrae candidates. An extension of this work using additional plate material and the same analysis procedures revealed several new RR Lyrae candidates, and a programme was set up to observe these objects spectroscopically in order to confirm that they were A stars. Of particular interest were the two faintest stars, R15 and R17 which, with magnitude $B \approx 20$, were apparently at an unexpectedly large distance from the galactic centre.

Photometric measures from 14 IIIaJ plates covering time scales from 1 day to several years are now available; the reduction procedures are described in detail elsewhere^{1,2}.

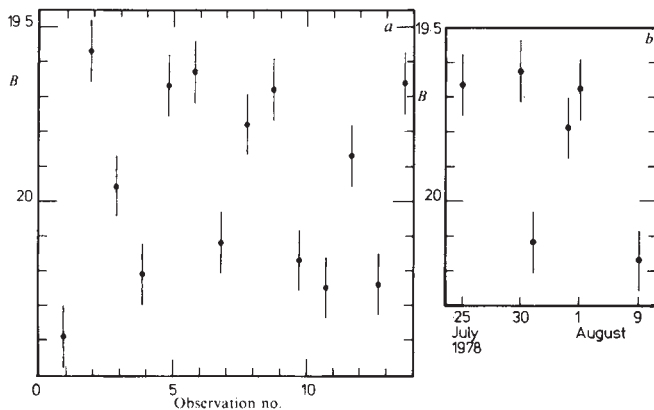


Fig. 1 *B* magnitudes for R15. *a* Shows all available magnitudes with observations equally spaced along the abscissa; *b* shows observations in 1978 correctly spaced in time.

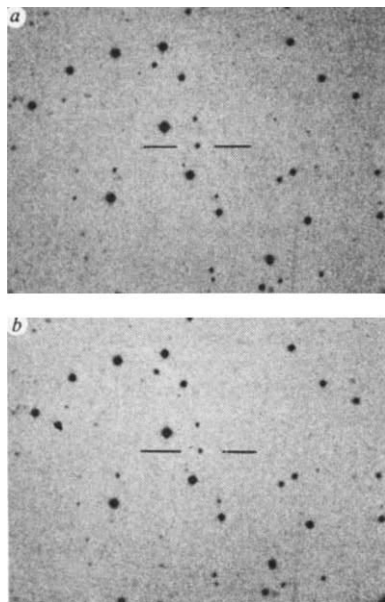


Fig. 2 R15 at: *a*, maximum from plate J3376; and *b*, minimum from plate J6229. North is upwards, east to the left. The field is $\sim 5 \times 4$ arc min.

Figure 1*a* shows measurements for R15, with observations equally spaced along the abscissa regardless of epoch. Figure 1*b* shows measurements correctly spaced in time over 3 weeks in 1978; the r.m.s. error for each observation is 0.09 mag. The distribution of magnitudes about the mean gives no indication of an eclipsing system, and shows that the object can vary over its full amplitude in 24 h. Visual inspection of the original plates confirms that R15 is indeed a variable; see Fig. 2*a,b* which shows it at maximum and minimum. The mean magnitude from 14 observations is $\langle B \rangle = 19.94$, with an amplitude of 0.82 mag. The variable behaviour is confirmed on a set of 25 IIIaF plates taken over 6 weeks in 1980, with $\langle R \rangle = 19.10$ giving a mean colour of $\langle B - R \rangle = 0.84$.

On the basis of these photometric observations, R15 was considered a candidate RR Lyrae and observed on the Anglo-Australian Telescope (AAT) with the Image Proportion Counting System (IPCS) during August 1982. The star was periodically checked until it appeared to be at maximum and then observed at a dispersion of $2 \text{ \AA}$ per pixel for four 1,000-s integrations. Figure 3 shows the resulting spectrum without flux calibration to allow a better judgment of the signal-to-noise ratio. The object is an A star with the Balmer series in absorption from $H\beta$ to $H\xi$ clearly visible, although there is no detectable Ca K line. The spectrum was not taken with the intention of measuring the radial velocity, however by chance shortly after observation it was compared with that of a brighter star

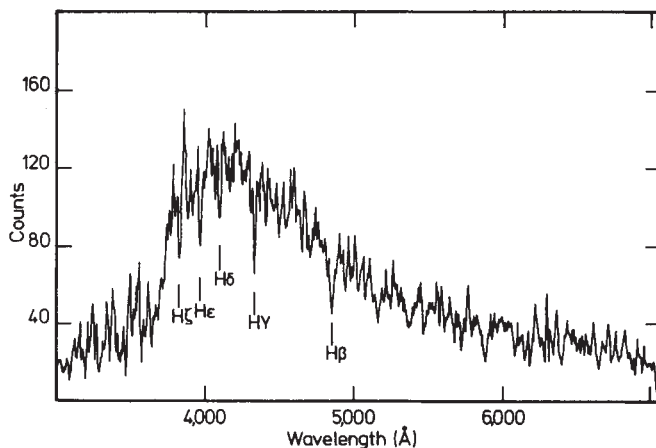


Fig. 3 The spectrum of R15 observed on the AAT with the IPCS. The Balmer series are marked.

by aligning the night sky lines. It was immediately obvious that the radial velocities differed by several hundred kilometres per second.

Due to the faintness of the object, the spectrum is not good enough to obtain an accurate radial velocity. However, two different approaches to the analysis have yielded reasonable agreement, with an error that is small enough for present purposes. The first method used the SPICA reduction package on Starlink³ to measure the wavelength of individual lines. This gave a radial velocity of $-418 \pm 129 \text{ km s}^{-1}$ where the error was derived from the scatter among the measures of the five lines used.

To get better use of all the information in the spectrum, the observations were re-analysed using the ESP system developed by B. D. Kelly and cross-correlated with spectra of two bright RR Lyrae stars, R07 and R22, whose radial velocities could be determined to $\pm 20 \text{ km s}^{-1}$ from their individual lines. The cross-correlation of R15 with these two stars gave a mean radial velocity for the five measures of $-483 \pm 10 \text{ km s}^{-1}$. As this agreement seemed fortuitously good, the cross-correlation was performed with three additional and rather fainter RR Lyrae objects. This gave a mean radial velocity of $-494 \pm 27 \text{ km s}^{-1}$ for the five measurements. Correcting for the pulsation velocity of the star, which was at maximum light when observed, reduces this⁴ by 29 km s^{-1} to $-465 \pm 27 \text{ km s}^{-1}$.

The absolute magnitude of halo RR Lyraes has long been a matter of debate, with current estimates^{5,6} for $\langle M_B \rangle$ ranging from 0.9 to 1.4. Due to the value for comparison purposes of the NGP survey of Butler *et al.*, we shall adopt their value of $\langle M_B \rangle = 0.9$ and their distance to the galactic centre, $R_0 = 8.7 \text{ kpc}$. By using the value of $B = 19.94$ cited above this gives a distance for R15 of 64 kpc from the Earth. The RA and dec. of R15 are $\alpha = 21 \text{ h } 23 \text{ min } 54.6 \text{ s}$, $\delta = 44^\circ 57' 01''$ (1950) with galactic coordinates $l^\parallel = 356^\circ$, $b^\parallel = -46^\circ$. This implies a distance above the plane $z = 45 \text{ kpc}$, a distance from the galactic centre $R = 59 \text{ kpc}$, and that the correction to the radial velocity due to the solar motion is negligible. If the star is in a bound orbit round the Galaxy, combining this distance with the radial velocity gives a lower limit to the mass of the Galaxy of

$$M_G > 1.4 \times 10^{12} M_\odot$$

The formal uncertainty in this value due to errors in the radial velocity is $\pm 11\%$. The uncertainty from the value assumed for the absolute magnitude is due both to intrinsic dispersion in RR Lyrae mean luminosities, and to the adoption of a possibly incorrect mean value. Estimates in the literature^{5,6} typically are in the range ± 0.15 to $\pm 0.25 \text{ mag}$; adopting the latter value gives an uncertainty in both the distance and the mass of $\pm 12\%$. We thus take the overall uncertainty in the mass limit as being $\pm 16\%$.

The question of whether the star is in a bound orbit is not easy to answer definitively. It is possible that R1 is a truly

intergalactic RR Lyrae lying in the path of the Galaxy or that it has been ejected towards us from a local group system. The classification of the star as an RR Lyrae is perhaps also debatable. A proper light curve, although extremely difficult to obtain, would be definitive. However, the present observations strongly suggest an RR Lyrae star, and do not seem consistent with any other known type of variable.

Several different dynamical systems have been used recently to determine the mass of the Galaxy. Einasto and Lynden-Bell⁶, from motions of the Galaxy, M31 and other outlying local group members, obtained a mass for the whole system of $3-6 \times 10^{12} M_{\odot}$. Hartwick and Sargent⁹, using globular clusters and local group members which are satellites of the Galaxy, obtained a mass out to 61 kpc of $8 \times 10^{11} M_{\odot}$. Frenk and White¹⁰ developed an improved analysis procedure which they applied to the globular cluster system within 33 kpc. Their favoured model gave a mass of $5.3 \times 10^{11} M_{\odot}$ out to 33 kpc, and when extrapolated to 60 kpc gave $1.3 \times 10^{12} M_{\odot}$. The drawback of all statistical dynamical mass estimates is that without complete knowledge of the velocity vectors of members of the system, certain assumptions must be made about the velocity ellipsoid, which inevitably introduces uncertainty into the results. The mass limit $M_G > 1.4 \times 10^{12} M_{\odot}$ given here depends only on the assumption that the star is bound to the Galaxy. The limit is consistent with the mass derived by Einasto and Lynden-Bell⁶ for the local group as a whole, and favours the higher mass determinations of Frenk and White¹⁰ for the Galaxy itself. It also directly and independently confirms the existence of a massive halo for the Galaxy.

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1. Hawkins, M. R. S. *Nature* **293**, 116-120 (1981).
2. Hawkins, M. R. S. *Mon. Not. R. astr. Soc.* **202**, 511-585 (1983).
3. Disney, M. J. & Wallace, P. T. *Q. J. R. astr. Soc.* **23**, 485-504 (1982).
4. Clube, S. V. M., Evans, D. S. & Jones, D. H. P. *Mem. R. astr. Soc.* **72**, 101-184 (1969).
5. Oort, J. H. & Plaut, L. *Astr. Astrophys.* **41**, 71-86 (1975).
6. Clube, S. V. M. & Dawe, J. S. *Mon. Not. R. astr. Soc.* **190**, 591-610 (1980).
7. Butler, D., Kinman, T. D. & Kraft, R. P. *Astr. J.* **84**, 993-1004 (1979).
8. Einasto, J. & Lynden-Bell, D. *Mon. Not. R. astr. Soc.* **199**, 67-80 (1982).
9. Hartwick, F. D. A. & Sargent, W. L. W. *Astrophys. J.* **221**, 512-520 (1978).
10. Frenk, C. S. & White, S. D. M. *Mon. Not. R. astr. Soc.* **193**, 295-311 (1980).

Circumvention of Parker's bound on galactic magnetic monopoles

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There is a possibility that a magnetic monopole has been observed by Cabrera¹. The monopole density implied by the observation appears to violate bounds on the density of such particles derived from the total mass density of the Universe and from the existence of galactic magnetic fields. We show here that the observation is not inconsistent with these bounds if the monopoles and antimonopoles are bound into positronium like states with principal quantum n high enough so that the Earth's magnetic field will break them apart, but small enough so that the weaker galactic magnetic field will not. We determine a range of values for n and show that lifetimes for such bound states are longer than the current age of the Universe.

The size of Cabrera's detector and the duration of his experiment would imply a monopole density, η , in our neighbourhood of the order of $10^{-16} [m_M/(10^{16} \text{ GeV}/c^2)]^{1/2} \text{ cm}^{-3}$. If such monopoles have masses of the order of $m_M \sim 10^{16} \text{ GeV}$, as

expected in some grand unified theories^{2,3}, then consistency with the upper bound on the mass of the Universe requires that η be as high as 10^{-16} cm^{-3} on scales of at most galactic size. The intergalactic density must be less than $\eta_{IG} \sim 10^{-24} \text{ cm}^{-3}$.

Parker^{4,5}, and Bludman and Ruderman⁶, have put an upper limit of 10^{-26} cm^{-3} on the density, η_G , of galactic monopoles by considering the effects of monopoles on the galactic magnetic field. For values of $\eta_G > 10^{-26} \text{ cm}^{-3}$ the monopole population takes energy out of galactic magnetic fields too rapidly for the fields to be maintained at their observed values of $B_G \sim 10^{-6} \text{ G}$. Furthermore, since the fields extend over distances of the order of 300 light years, interaction of the monopoles with B_G would, for $m_M < 10^{19} \text{ GeV}$, result in their ejection from the Galaxy⁵ thereby implying inconsistency between the observation and the intergalactic density limit.

There is thus a discrepancy of the order of 10^{10} between the recent possible observation of a monopole density $\eta \sim 10^{-16} \text{ cm}^{-3}$ and the upper limit of $\eta \sim 10^{-26} \text{ cm}^{-3}$ allowed by galactic magnetic fields. Our suggested mechanism for removing this discrepancy is that monopole-antimonopole ($M-\bar{M}$) bound states of low enough principal quantum number would not be dissociated by, and hence would not take energy from, intergalactic magnetic fields. They would, however, be dissociated by the Earth's magnetic field, for high enough principal quantum number. Hence they would be separated near the Earth as was possibly observed¹.

The Bohr formula for the $M-\bar{M}$ bound state energy is

$$E_n = \frac{g^4 m_M}{4n^2} = \frac{g^2}{r_n} \quad (1)$$

where the monopole charge g is given by $ge = 1/2$. Setting E_n equal to gBr_n gives the lower bounds on the principal quantum number n for which the $M-\bar{M}$ bound states will be dissociated by the magnetic field B

$$n \sim 3 \times 10^5 m_M^{1/2} B^{-1/4} \quad (m_M \text{ in GeV}, B \text{ in G}) \quad (2)$$

Thus for $m_M \sim 10^{16} \text{ GeV}$, the $M-\bar{M}$ bound state will be dissociated by the 1 G field of the Earth, but not by the 10^{-6} G intergalactic field, if n is given by

$$3 \times 10^{13} \leq n \leq 10^{15} \quad (3)$$

The lifetime τ_R for such bound states to decay by spontaneous emission of radiation can be found from the Bohr model

$$\frac{dE}{dt} = \frac{2}{3} g^2 a^2 \quad \text{with } a = \frac{v^2}{r}$$

It is given by

$$\tau_R \approx \frac{n^6 \hbar}{m_M g^{10}} \quad (4)$$

After a time τ_R an $M-\bar{M}$ bound state will have reached a low enough value of n for the pair to annihilate into ordinary light particles. For $n = 3 \times 10^{13}$ and $m_M = 10^{16} \text{ GeV}$, τ_R is 10^{23} yr —far longer than the present age of the Universe.

$M-\bar{M}$ bound states will be dissociated (or excited) by bound state-bound state collisions. The lifetime τ_c for such collisions is given by

$$\tau_c \sim (\eta \sigma v)^{-1} \quad (5)$$

We take the cross-section as $\sigma \sim r_n^2$ and v as the virial velocity of masses bound to the galaxy ($v \sim [GM_{\text{galaxy}}/R_{\text{galaxy}}]^{1/2} \sim 10^{-3} c$). Using $\eta = 10^{-16} \text{ cm}^{-3}$ gives

$$\tau_c \sim 10^{38} \frac{m_M^2}{n^4} \quad (6)$$

For $m_M = 10^{16} \text{ GeV}$ and $n = 3 \times 10^{13}$, τ_c is 10^{17} s which is of the order of the age of the Universe. A slight decrease in the average n would put τ_c well above the age of the Universe but would require a larger magnetic field for dissociation. Combining equation (6) with equation (2) gives $B \sim 5 \text{ G}$ for τ_c equal to the age of the Universe. We consider this discrepancy mildly

discouraging but, with the rough approximations involved (particularly for the cross-section) and the high powers of n , not decisive. These considerations, in any event, indicate a likely value for n in the range 10^{13} – 3×10^{13} .

Because of the large mass of grand unified monopoles, after dissociation by the Earth's magnetic field they do not separate rapidly. With a velocity of the order of $10^{-3}c$ it is easy to see that in a distance L they will separate by about $\Delta X \sim gBL^2/m_M$. Thus 300 km at 1 G are necessary to achieve a 1 cm separation. This probably rules out use of laboratory fields to separate pairs of $n < 3 \times 10^{13}$ but shows that separations > 1 cm are to be expected after passage through the Earth's field.

There exists, from the radiation from neutron stars, a limit^{7,8} on the product of the density of monopoles times the cross-section for monopoles to catalyse proton decay^{9–11}. If the density is 10^{-16} cm^{-3} as implied by ref. 1 then the cross-section must be at least as small as a typical weak interaction cross-section, 10^{-40} cm^2 . A cross-section of this order has been advocated by Preskill and Wilczek at the Nuffield Foundation workshop¹². If, on the other hand, the cross-section is of the order of a typical strong interaction cross-section, 10^{-27} cm^2 (refs 9, 10), then the density of monopoles must be $< 10^{-32} \text{ cm}^{-3}$, much less than the Parker bound; the possibility of bound monopole–antimonopole states does not address this potential inconsistency.

From the value of n and the assumption that the monopole temperature at bound state formation must have been of the order of $T_M \sim E_n \sim 10^{-4} \text{ MeV}$ we can fix approximate bounds on the time of bound state formation. If monopoles at that time were still in thermal equilibrium with radiation and light particles, and did not dominate the mass of the Universe, formation must have occurred at about 10^8 s . If, at the other extreme, monopoles decoupled from light particles at $T \sim 1 \text{ GeV}$ and did dominate the mass density of the Universe, this temperature was reached at $t \approx 30 \text{ s}$.

Preskill¹³ has pointed out that, in the standard model of the big bang, the primordial density of grand unified monopoles is only reduced to $\eta = 10^{-10} T^3$ for $T > 10^{10} \text{ GeV}$. At later times, $T < 10^{10} \text{ GeV}$, several annihilation processes have been studied. These include: (1) $M + \bar{M} \rightarrow \gamma + \gamma$ (ref. 13); (2) $M + \bar{M} \rightarrow (M\bar{M}) + M$ where the parenthesis indicate a bound state¹⁴; (3) processes (1) and (2) enhanced by clumping^{14,15}; and (4) magnetic Coulomb binding of neighbouring $M, \bar{M}$ pairs that are thermally decoupled from light particles with the expansion of the Universe¹⁶. This process is based on the fact that the kinetic energy drops as R^{-2} while the potential energy of pairs drops only as R^{-1} ; it was studied in ref. 16 for e^+e^- pairs in an expanding ($k=0$) universe. (There are, as pointed out in ref. 14, significant parallels between late ($k=0$) universe e^+e^- processes and early universe $M-\bar{M}$ processes.) None of these four processes reduces η sufficiently without diluting the nucleon photon ratio below that observed today¹⁴.

The same mechanisms that fail to reduce η satisfactorily also fail to explain binding of $M-\bar{M}$ pair at times on the order of 10^2 – 10^8 s discussed above. It is clear, however, that if both the standard model of the Universe and a grand unified model for particle interactions which includes the existence of magnetic monopoles are correct, such mechanisms must exist.

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1. Cabrera, B. *Phys. Rev. Lett.* **48**, 1378–1381 (1982).
2. 't Hooft, G. *Nucl. Phys.* **B79**, 276–284 (1974).
3. Polyakov, A. *Pis'ma Zh. eksp. teor. Fiz.* **20**, 430–433 (1974); [*JETP Lett.* **20**, 194–195 (1974)].
4. Parker, E. *Astrophys. J.* **160**, 383–404 (1970).
5. Turner, M. S., Parker, E. & Bogdan, T. *Phys. Rev.* **D26**, 1296–1305 (1982).
6. Bludman, S. A. & Ruderman, M. A. *Phys. Rev. Lett.* **36**, 840–843 (1976).
7. Kolb, E. W., Colgate, S. A. & Harvey, J. A. *Phys. Rev. Lett.* **49**, 1373–1375 (1982).
8. Dimopoulos, S., Preskill, J. P. & Wilczek, F. *Phys. Lett.* **119B**, 320–322 (1982).
9. Callan, C. G. *Phys. Rev.* **D25**, 2141–2146 (1982).
10. Rubakov, V. A. *Pis'ma Zh. eksp. teor. Fiz.* **33**, 658–660 (1981); [*JETP Lett.* **33**, 644–646 (1981)].
11. Wilczek, F. *Phys. Rev. Lett.* **48**, 1146–1149 (1982).
12. Barrow, J. D. & Turner, M. S. *Nature* **298**, 801–808 (1982).
13. Preskill, J. P. *Phys. Rev. Lett.* **43**, 1365–1368 (1979).
14. Dicus, D. A., Page, D. N. & Teplitz, V. L. *Phys. Rev.* **D26**, 1306–1316 (1982).
15. Goldman, T., Kolb, E. W. & Toussaint, D. *Phys. Rev.* **D23**, 867–875 (1981).
16. Barrow, J. D. & Tipler, F. J. *Nature* **276**, 453–459 (1978).

Some characteristics of a rapidly rotating magnetized neutron star

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The recently discovered^{1,2} 1.5-ms pulsed radio source PSR1937+214 could be close to the onset of rotational instabilities if it is envisaged to be a rotating neutron star which gives interesting lower limits for neutron star masses. We examine here the implications of polar gap models for such a rapidly rotating magnetic neutron star. It appears, on the basis of such models, that a higher fraction of high-frequency microwave radiation compared with the Crab pulsar is an expected result. On the other hand, the outer gap discharge models invoked for longer period pulsars yield a large amount of high-energy radiation which is not observed. The high-energy cutoff in the γ -ray region evaluated from assumed values of the magnetic field inside the light cylinder will be around 1,000 GeV.

We examine the characteristics of electromagnetic emission from a rapidly rotating magnetized neutron star. The various scenarios in which such an object could be formed have been discussed elsewhere^{3–5}.

The study of differentially rotating, inviscid polytropes with specified angular momentum distribution shows that the constructed sequence of models⁶ closely resemble the Maclaurin sequence. Given the total mass and angular momentum and a prescribed distribution of angular momentum, a sequence of axisymmetric models in a state of permanent rotation can be constructed that depends on the parameter $\tau = (\text{kinetic energy of rotation})/(\text{gravitational potential energy})$. A point of bifurcation is reached near $\tau = 0.138$ where non-axisymmetric figures of equilibrium branch off and models are expected to be secularly unstable in the presence of gravitational radiation. The exact value of τ depends to some extent on the model considered, but it is very near the bifurcation point $\tau_b = 0.1375$ of a uniformly rotating, homogeneous spheroid. Beyond τ_b , all Maclaurin spheroids slowly evolve to the Jacobi sequence (with triplanar symmetry) when viscous friction is taken into account. In the case of Maclaurin spheroids with both viscosity and gravitational radiation, the onset of secular instability can be delayed beyond $\tau = \tau_b$ to a point determined by the relative strengths of the dissipative forces^{7,8}. One specific choice of the viscosity causes the Maclaurin sequence to be secularly stable even up to the onset of a dynamical instability at $\tau = \tau_i = 0.2738$.

A rapidly rotating fluid star can therefore be expected to be stable at least for $\tau < 0.1375$. For a uniformly rotating homogeneous spheroid, this limit corresponds to

$$\frac{\Omega^2}{2\pi G \bar{\rho}} \approx 0.18 \quad (1)$$

which for a period of 1.558 ms would imply a mean stellar density $\bar{\rho} = 2.4 \times 10^{14} \text{ g cm}^{-3}$. Note that for a variety of equations of state, neutron star models beyond a certain stellar mass permit densities well beyond this. For example, with a recent high density equation of state due to Friedman and Pandharipande⁹ which incorporates the effects of two and three nucleon interactions, the mean density reached in a non-rotating star of mass $1.4 M_\odot$ is $4.1 \times 10^{14} \text{ g cm}^{-3}$. Thus for such an equation of state, a $1.4 M_\odot$ star is rotationally stable even for a fast pulsar with a period of 1.56 ms. However, such fast rotation puts an interesting lower bound for neutron star mass (for a given equation of state) obtained from the criterion for rotational stability, since the mean density is determined by the stellar mass and equation of state. The few cases where neutron star masses have been estimated through a combination of X-ray and radio observations indicate a narrow spread around

$1.4 M_{\odot}$. Thus, the theoretical lower bounds on the masses of stable neutron stars, for an assumed equation of state and with rotational speeds as high as the millisecond pulsar, should not be inordinately larger than $1.4 M_{\odot}$. Recently B. Datta and A. Ray (personal communication) have constructed models for critically rotating neutron stars with a view to narrowing down the admissible equations of state.

In the early models due to Pacini^{10,11} and Ostriker and Gunn¹² the secular increase in the period ($\dot{P}$) was attributed to the loss of rotational energy due to magnetic dipole radiation with a possible contribution due to gravitational radiation. The canonical magnetic field associated with pulsars is $\sim 10^{12}$ G, but from the measured value of $\dot{P} \sim 1.2 \times 10^{-19} \text{ s s}^{-1}$ (refs 13, 14) for the new pulsar, the magnetic field at the neutron star surface, implied by the magnetic dipole radiation, is $B \sim 5 \times 10^8$ G. The radio frequency pulses from pulsars are believed to be due to magnetic field induced discharge processes near the polar cap of the neutron star (see the Ruderman Sutherland model¹⁵). The total power dissipated in such 'polar gaps' is roughly a thousand times the amount of radiation detected in the radio band for the Crab pulsar and is given as:

$$\dot{E} \sim 10^{30} B_{12}^{6/7} s_6^{4/7} P^{-15/7} \text{ erg s}^{-1} \quad (2)$$

Here B_{12} is the field in 10^{12} G, s_6 is the radius of curvature of the magnetic field in 10^6 cm and P is in s.

Using the appropriate numbers for the Crab pulsar and the PSR1937+214, this expression gives very similar numbers ($3.8 \times 10^{33} \text{ erg s}^{-1}$ for Crab and $1.55 \times 10^{33} \text{ erg s}^{-1}$ for PSR1937+214) for the polar gap particle luminosity $\dot{E}$. In this connection, note that the reported total radio flux from the new pulsar² is also within a factor of two of the Crab pulsar (subject to similar beaming factors). As the period and (inferred) magnetic field of the new pulsar are such as theoretically to allow for pair-production discharges, the correlation in radio flux indicates that the discharge-induced electromagnetic radiation mechanisms must play a part even for such widely different parameters as between the fastest and the pulsars like the Crab. The new pulsar has a radio-frequency spectrum that is flatter than the Crab pulsar. The light cylinder radius of PSR1937+21 is much smaller than that of the slower pulsars. Consequently, the radius to frequency mapping implies that the frequency of the emitted radio radiation, which is at the local plasma frequency, is higher for closer radial distances of the emission zone. Thus, the new pulsar should have a greater fraction of high-frequency radio radiation and hence a flatter spectrum.

It is argued¹⁶ from the considerations of Crab and Vela pulsars that the region of production of high-energy radiation is different from the polar cap of the neutron star and is attributed to the 'outer gaps'¹⁷. The total power output of 'radiation reaction limited outer gaps' is weakly dependent on the stellar magnetic field but is a sensitive function of the location of the accelerating region of the e^+e^- plasma and the pulsar period:

$$\dot{E}_{\text{O.G.}} \sim 3 \times 10^{32} r_7^{16/5} s_7^{3/5} P^{-2.1} B_{s,12}^{-1/10} \text{ erg s}^{-1} \quad (3)$$

With usual assumptions regarding outer gap parameters and the field line curvature radius of the order of the stellar radius, this leads to a energy production rate for the 1.5 ms pulsar:

$$\dot{E}_{\text{O.G.}} \sim 8 \times 10^{36} \text{ erg s}^{-1}$$

Even if a few per cent of this power appeared in the X-ray band, the estimated flux at Earth at a distance of ~ 3 kpc would be $\sim 10^{-10} \text{ erg s}^{-1}$. This should have been certainly detected by the Einstein observatory. [On the other hand if the X rays are generated in the polar gap only, the flux estimated similarly ($\sim 10^{-15} \text{ erg cm}^{-2} \text{ s}^{-1}$) would be below the threshold of the Einstein IPC.] Aside from these severe observational restrictions against high-altitude accelerating zones, there are independent theoretical reasons, based on magnetospheric structures to believe that these discharging regions (outer gaps) may not work for as fast a pulsar as the 1.5 ms object.

On the basis of the observed spin down rate and the expected power output (2) from gap discharge models, one expects a lot

more radiation from the new pulsar than is observed in the radio. It is possible that the pulsar can be radiating in the high-energy regions including at γ -ray energies just as in the case of Crab and Vela. In such a case one can consider what the theoretical upper limit of the photon energy in the pulsed radiation from this pulsar would be. Most mechanisms of electromagnetic radiation from pulsars place the region of emission well within the light cylinder. Even with a smaller surface dipole field of 5×10^8 G, the dipole field at the light cylinder is roughly comparable with the one at Crab pulsar's light cylinder. Now, a γ -ray photon of energy ϵ_{γ} (in eV) travelling at an angle θ with respect to the magnetic field B (in gauss) produces an e^+e^- pair if the following condition due to Sturrock¹⁸ is satisfied:

$$\epsilon_{\gamma} B \sin \theta \geq 10^{18.6} \quad (4)$$

Thus a photon of energy ϵ_{γ} will be removed from the beam if the magnetic field is strong enough to allow photo pair production. With higher multipoles present along with the dipole component, the magnetic field at the light cylinder could be of the order of 4×10^6 G. This would imply a cutoff in the γ -ray spectrum at energy $\epsilon_{\gamma} = 1,000$ GeV and thus would make the Čerenkov shower detectors (which have a threshold generally above 1,000 GeV) unsuitable. But this upper limit is likely to be a conservative estimate. If the pulsed radiation is to be substantially beamed at the pulse period, the region of production of high-energy radiation is likely to be well within the light cylinder in the standard models where the magnetic field would be closer to the surface value. In such a case, the cutoff energy could even be lowered to 10–50 GeV. Although the COS B catalogue does not list any steady source of γ rays within $\sim 3^\circ$ away from the position of the radio pulsar, nevertheless, a careful search for pulsed γ -ray emission from the position of this new pulsar by a future satellite-borne telescope is called for. If this proves to be fruitful and indicates a cutoff in the spectrum in the above mentioned range, then one would have independent information regarding the magnetic field strength in the region of high-energy emission.

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- Heiles, C. *et al.* *Nature* **300**, 399 (1982).
- Backer, D. C., Kulkarni, S. R., Heiles, C., Goss, W. M. & Davis, M. M. *Nature* **300**, 615–618 (1982).
- Radhakrishnan, V. & Srinivasan, G. *Curr. Sci.* **51**, 1096–1099 (1982).
- Alpar, M. A. *et al.* *Nature* **300**, 728–729 (1982).
- Henrichs, H. F. & vanden Heuvel, E. P. J. preprint (University of Amsterdam, 1983).
- Bodenheimer, P. & Ostriker, J. P. *Astrophys. J.* **180**, 159–169 (1973).
- Lindblom, L. & Detweiler, S. L. *Astrophys. J.* **211**, 565–567 (1977).
- Detweiler, S. L. & Lindblom, L. *Astrophys. J.* **213**, 193–199 (1977).
- Friedman, B. & Pandharipande, V. R. *Nucl. Phys. A* **361**, 502–520 (1981).
- Pacini, F. *Nature* **216**, 567–568 (1967).
- Pacini, F. *Nature* **219**, 145–146 (1968).
- Ostriker, J. P. & Gunn, J. E. *Astrophys. J.* **157**, 1395–1417 (1969).
- Ashworth, M., Lyne, A. G. & Smith, F. G. *Nature* **301**, 313–314 (1983).
- Backer, D. C., Kulkarni, S. R. & Taylor, J. H. *Nature* **301**, 314–315 (1983).
- Ruderman, M. A. & Sutherland, P. G. *Astrophys. J.* **196**, 51–72 (1975).
- Ruderman, M. A., *Pulsars*, IAU Symp. No. 95, 254 (1981).
- Cheng, A., Ruderman, M. A. & Sutherland, P. G. *Astrophys. J.* **203**, 209–217 (1976).
- Sturrock, P. A. *Astrophys. J.* **164**, 529–556 (1971).

Comparison of CO₂ measurements by two laboratories on air from bubbles in polar ice

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The CO₂ content of air enclosed in bubbles in polar ice has been reported by our two laboratories (in Grenoble and Bern) to be representative of the atmospheric CO₂ concentration at the time the ice was formed^{1,2}. Such ice core studies indicate

lower concentrations in ice formed at the end of the ice age, around 18,000 yr BP, and several explanations have been proposed for such a change^{3,4}. Both laboratories are currently measuring various ice cores in order to determine the pre-AD 1850 CO₂ level in the atmosphere, which relates to the partitioning of anthropogenic CO₂ among the atmospheric, biospheric and oceanic reservoirs. The two laboratories use different ice cores and different analytical procedures and we, therefore, need to know to what extent the measurements are quantitatively comparable. We present here the results of a comparison between the two laboratories based on measurements from the same ice core sections, which indicate that the measurements can be compared with great confidence. The results suggest that the mean CO₂ level recorded by Antarctic ice for the period 800–2,500 yr BP is about 260 p.p.m.v.

In order to preclude any gas fractionation linked with surface summer melting, ice formed in very cold conditions is the most suitable for determining CO₂ concentrations representative of the atmosphere. We selected for the present intercalibration a section of a 905-m core⁵ drilled at Dome C (Antarctica, 74°42' S, 124°04' E) where the mean annual temperature is –54 °C. Because gas extraction from ice is a destructive process it is not possible to make comparative measurements on the same ice samples, but to reduce sources of variability, an uncracked core section with a fairly homogeneous bubble distribution was chosen, and split vertically into two halves. The selected section, about 30 cm long, was taken from a depth of 132.9 m below the ice sheet surface. For cross-checking, a smaller section at a depth of 401.5 m from the core drilled to bedrock at Byrd Station⁶ (Antarctica, 80°01' S, 119°32' W) was used. The quality of this core was worse than that of the Dome C section and a few fractures were observed. The air bubbles in ice become progressively isolated from the atmosphere during the transformation of snow into ice, and the gas analysed in any ice sample corresponds to a certain time interval of the past. The maximum extent of this interval for the selected Dome C section is estimated to be 800–2,500 yr BP and that for the Byrd section is included within the preceding one.

Dry extraction methods avoiding melting must be used to obtain reliable CO₂ concentrations of the air trapped in bubbles in ice. In both laboratories the samples are cut with a bandsaw in a cold room at about –15 °C, as close as possible to the centre of the core (to avoid surface contamination). A typical cutting scheme is shown in Fig. 1. At Grenoble, the ice sample (about 35 g) is crushed under vacuum in a stainless-steel container⁷. The stainless-steel container (maintained at about –40 °C) is then connected to an extraction line (Fig. 2) which is first evacuated for 30 min (dynamic pressure $\leq 10^{-2}$ torr). The extracted gases are then expanded into the line and the gas in the sampling loop is injected into the gas chromatograph. The pressure of the gas in the sample loop was measured first with an absolute aneroid manometer and then with a capacitive pressure sensor. The calibration of the analytical system including the extraction line and the gas chromatograph, but excluding the stainless-steel container with the ice sample, is achieved using a standard mixture of CO₂ in nitrogen and oxygen (prepared by Air Liquide). The accuracy (2σ) of the measurements is evaluated from the standard deviation of the residuals corresponding to the calibration regression. This accuracy is $\leq 10\%$ when using the aneroid manometer and $\leq 3\%$ when using the capacitive pressure sensor. The difference in accuracy is mainly due to a difference in the internal volume of the pressure measuring systems and consequently in the amount of gas injected into the gas chromatograph.

At Bern, small ice cubes (1–2 g) are crushed by a needle matrix in an evacuated container at –20 °C. The container is connected to a cold trap (–80 °C) and the absorption cell of an IR laser spectrometer (Fig. 2). Before crushing, the entire system is evacuated for 10 min (dynamic pressure $< 10^{-3}$ torr). After fast crushing (within 1 s), the gas originally trapped in the bubbles expands into the absorption cell where the CO₂ concentration is measured repeatedly over an interval of a few

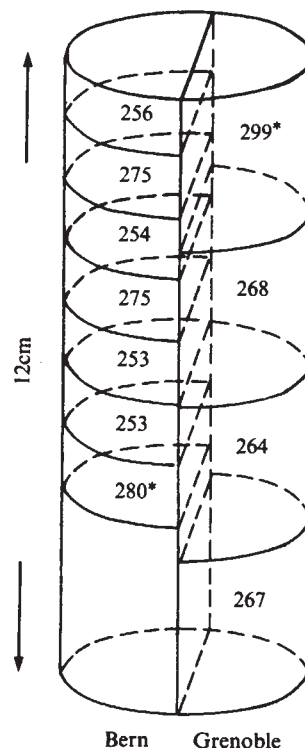


Fig. 1 Cutting scheme of the Byrd core section used in this study. The CO₂ concentration corresponding to each slice is indicated in p.p.m.v. * Presence of internal cracks.

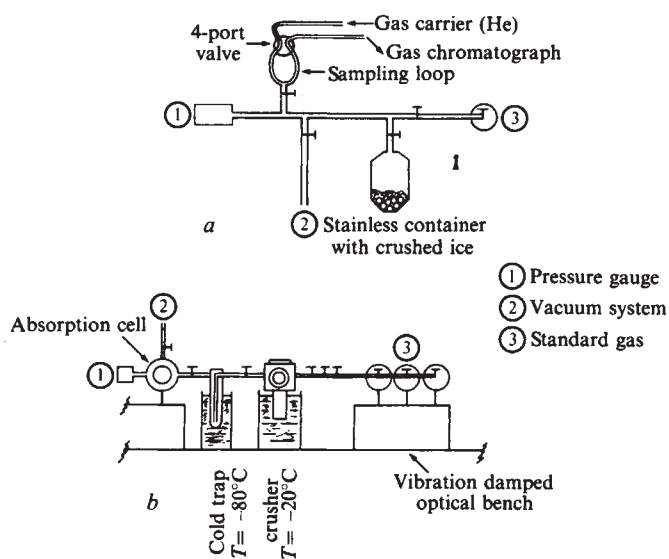


Fig. 2 Scheme of the analytical sets used in Grenoble (a) and in Bern (b).

minutes⁸. The system is calibrated after each measurement by injecting a standard gas, prepared according to the WMO 1974 provisional scale, into the crusher and absorption cell in similar conditions as the gas extracted from ice bubbles. The accuracy (2σ) evaluated experimentally from these calibrations is $\leq 3\%$.

The main characteristics of both experimental procedures are compared in Table 1 which indicates important differences between the methods concerning the sample size, the temperature and time of crushing, and the surface area of the crushed ice.

To ensure the comparability of the measurements performed in the two laboratories we have measured the CO₂ concentration of the standard used at Grenoble with the Bern analytical system. The three measurements performed give the following

Table 1 Comparison between the two analytical methods

	Grenoble	Bern
Dry extraction device	Metal ball crusher	Needle crusher
Ice sample size	30–40 g	1–2 g
Crushing time	90 s	1 s
Extraction efficiency	90% $\pm$ 5%	75% $\pm$ 15%
Surface area of the crushed ice	0.6 m ² g ⁻¹	2 $\times$ 10 ⁻² m ² g ⁻¹
Crushing temperature	-43 $\pm$ 3 °C	-20 $\pm$ 3 °C
Extraction temperature	-37 $\pm$ 3 °C	-20 $\pm$ 3 °C
Time interval between crushing and analysis	30 min	30 s
CO ₂ detection system	Gas chromatograph with high frequency helium detector	IR laser spectrometer
Volume of gas used for the analysis (STP)	0.01 cm ³ (using the aneroid manometer) 0.06 cm ³ (using the capacitive pressure sensor)	0.04 cm ³
Standard gases CO ₂ in N ₂ + O ₂	224 $\pm$ 4 p.p.m.v. (Air Liquide)	321 p.p.m.v., 342 p.p.m.v. 384 p.p.m.v. $\pm$ 0.1 p.p.m.v. (WMO provisional scale 1974)
Precision	10% (using the aneroid manometer) 3% (using the capacitive pressure sensor)	3%

results: 223.6, 224.4 and 222.9 p.p.m.v. with a mean value of 223.6 p.p.m.v. This is in very good agreement with the value provided by Air Liquide (224 $\pm$ 4 p.p.m.v.). The measurements of the two laboratories can also be compared with current CO₂ measurements performed on a global scale in the atmosphere, since the standards being used in Bern are prepared according to the WMO 1974 provisional scale by the Scripps Institution of Oceanography (USA).

The results of the measurements performed on the section of the Dome C core are shown in Fig. 3 and those corresponding to the Byrd core in Fig. 1. In Bern the core section is cut in slices representing an ice thickness of $\sim$ 1.5 cm in the ice sheet; two measurements were generally performed on each slice for the Dome C core (Fig. 3b) and one in the case of the Byrd core. At Grenoble each measurement is representative of an ice thickness of $\sim$ 3 or 4 cm; in the case of the Dome C core two complementary measurements have been performed, one representative of an ice thickness of $\sim$ 11 cm and the second of $\sim$ 7 cm (Fig. 2a). For comparison purposes, the Bern measurements on the Dome C core have been averaged over the same ice thickness (about 3 cm) as those measured in

Grenoble (Fig. 2a). The results thus obtained in both laboratories agree within the limits of experimental error. The average value over the entire core section is 258 p.p.m.v. in both cases. In the case of the Byrd core the comparison is more difficult due to the presence of fractures (Fig. 1). Two samples contained obvious fractures (one measured in Bern and one in Grenoble), and correspond with the highest CO₂ values (280 and 299 p.p.m.v.) measured on the section. If we disregard these two samples, the average CO₂ contents measured on the same ice thickness of the section are 263 p.p.m.v. (Bern) and 268 p.p.m.v. (Grenoble). The average values over the entire core section, excluding the fractured samples, are 261 p.p.m.v. (Bern) and 266 p.p.m.v. (Grenoble). These figures agree within the limits of experimental error.

The Bern measurements are performed on small samples (1–2 g of ice) on horizontal slices about 1.5 cm thick. As noted above, in the case of Dome C two measurements were generally performed on each slice. The scatter of the measured values corresponding to the same slice exceeds the experimental noise in 5 of the 15 slices (see Fig. 3b).

The comparative measurements therefore indicate that results obtained in both laboratories are the same, and that the different treatments of the samples do not lead to a difference in the measured CO₂ concentrations. The two extraction procedures involve different temperatures and durations of crushing and yield different sizes and shapes of crushed material. That the CO₂ results are independent of these factors indicates that interacting processes, such as adsorption, between CO₂ and pulverized ice in such crushing conditions are negligible. More generally, the fact that similar results are obtained by the two procedures adds confidence that the reported CO₂ concentrations do not depend on experimental conditions using a dry extraction method, and consequently are the true CO₂ concentrations of the air trapped in the bubbles. Furthermore, the good agreement between results obtained from a few grams of ice (Bern) and those obtained from a few tens of grams (Grenoble) indicates that the CO₂ concentrations in bubbles are homogeneous both vertically and horizontally on a scale of a few centimetres.

Some results obtained at Bern on small samples taken from the same 1.5-cm thick slice suggest that very local fluctuations (up to 25 p.p.m.v., see Fig. 3b) of the CO₂ concentrations can be present. The causes of such fluctuations are not clear. They could be linked to some heterogeneities occurring during the air occlusion process and also during and after ice drilling (for instance, the presence of small fractures which are difficult to observe visually). Nevertheless these fluctuations are not systematic and the measured mean values can be expected to be representative of the CO₂ content of the bubbles at the time they closed off from the atmosphere.

The measurements presented here are calibrated directly or indirectly with the standards prepared according to the WMO 1974 provisional scale. The absolute values can thus be com-

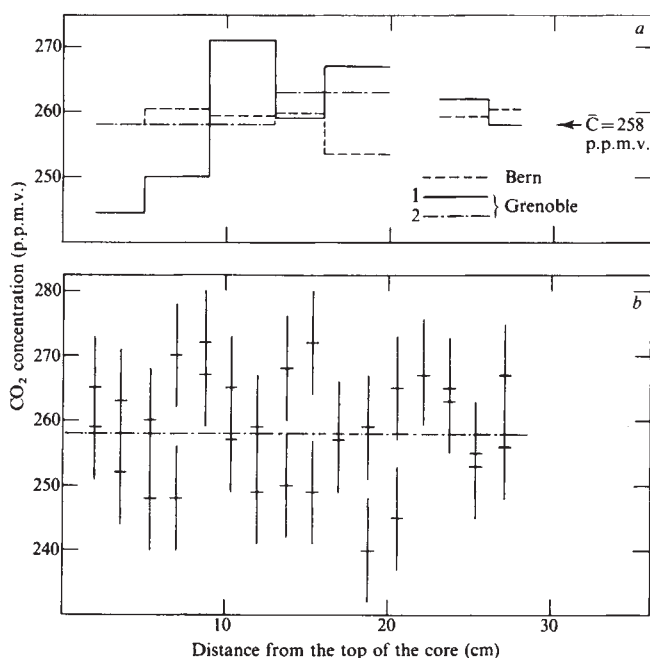


Fig. 3 CO₂ concentrations measured in Bern and Grenoble on a core section from Dome C (East Antarctica; depth: 132.9 m below the surface). *a*, Comparative results between the two laboratories; sets 1 and 2 were measured in Grenoble with different experimental precisions (set 1: 10%, set 2: 3%). *b*, Individual measurements performed in Bern on slices about 1.5 cm thick together with the estimated experimental error.

pared with the CO₂ content now observed in the atmosphere. Our results indicate both for Dome C (258 p.p.m.v.) and Byrd (261–266 p.p.m.v.) CO₂ concentrations much lower than the present-day atmospheric content (about 340 p.p.m.v.), and suggest a mean atmospheric value of about 260 p.p.m.v. for the period 800–2,500 yr BP. During this time interval anthropogenic influence on the atmospheric CO₂ level can be neglected. The value 260 p.p.m.v. is well within the range indicated by more indirect proxy data calculated from $\delta^{13}\text{C}$ records of tree-rings (see, for example, ref. 9). Our results are slightly but not significantly lower than the value of 271 p.p.m.v. suggested by measurements performed on a core section from North Central, Greenland, for the 'pre-industrial' period² and fluctuations in the atmospheric CO₂ level during this period of the Holocene cannot be discounted.

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1. Delmas, R. J., Ascencio, J. M. & Legrand, M. *Nature* **284**, 155–157 (1980).
2. Neftel, A., Oeschger, H., Schwander, J., Stauffer, B. & Zumburn, R. *Nature* **295**, 220–223 (1982).
3. Broecker, W. S. in *Climatic Variations and Variability: Facts and Theories*, 111–121 (ed. Berger, A.) (Reidel, Dordrecht, 1981).
4. Berger, W. H. *Naturwissenschaften* **69**, 87–88 (1982).
5. Lorius, C. & Donnou, D. *Cour. Cent. nat. Res. Scient.* **30**, 6–17 (1978).
6. Ueda, H. T. & Garfield, D. E. *Cold Regions Research and Engineering Laboratory Tech. Rep.* 231 (1969).
7. Raynaud, D., Delmas, R., Ascencio, J. M. & Legrand, M. *Ann. Glaciol.* **3**, 265–268 (1982).
8. Zumburn, R., Neftel, A. & Oeschger, H. *Earth planet. Sci. Lett.* **60**, 318–324 (1982).
9. Lorius, C. & Raynaud, D. in *Carbon Dioxide, a Text on Current Views and Developments in Energy/Climate Research*, 145–176 (eds Bach, W., Crane, H., Berger, A. & Longhetto, A.) (Reidel, Dordrecht, in the press).

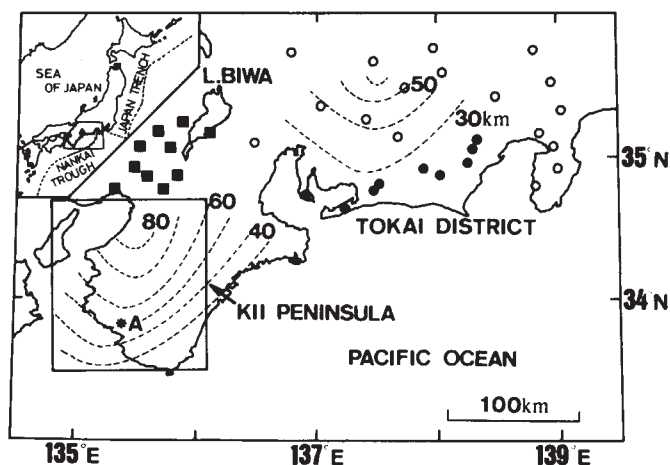


Fig. 1 Distribution of seismic stations (■, ○, ●) and depth contours of the subcrustal seismic zone at 10 km interval (broken lines). The focal region analysed in this study is enclosed by a solid rectangle. The epicentre of the event referred to Fig. 2 is also shown.

We studied 35 subcrustal earthquakes occurring beneath the Kii peninsula in 1979–82. Their epicentral region is enclosed by a solid rectangle in Fig. 1. The hypocentral parameters of these earthquakes are given by the Japan Meteorological Agency which determines focal depths at 10-km intervals. The Moho lies at a depth of ~30 km in this region¹³. Figure 1 also shows the locations of the three-component seismographic stations telemetered to the Kyoto University, the Nagoya University and the National Research Center for Disaster Prevention. Some of these stations show a distinctive pair of P and S later phases for subcrustal earthquakes at depths shallower than 60 km. No such phases are observed for deeper events. In Fig. 1, the relevant stations are distinguished by solid circles from other stations and are all located in the coastal region of the Tokai district. We suggest that these later phases are generated within the untransformed oceanic crust descending into the uppermost mantle.

Figure 2a shows an example for the P-wave seismograms in the coastal region. The records are for event A in Fig. 1. The focus lies at a depth of 50 km and the epicentral distance ranges from 150 to 300 km. The first arrivals with an apparent velocity of ~8.0 km s⁻¹ are too weak to read their exact onsets. A much stronger later phase follows with an apparent velocity of ~6.6 km s⁻¹. This phase is so distinct and so systematic that one might interpret it as being due to some critical reflection within the Earth. The observed apparent velocity is, however, too low to permit such an interpretation. On the corresponding S-wave seismograms recognizable phases are hardly seen near the S arrival times calculated from a standard travel time table. An outstanding S phase arrives significantly late with an apparent velocity of ~3.9 km s⁻¹. Table 1 summarizes the results of such observations. The apparent P velocities obtained are 7.9–8.1 km s⁻¹ for the initial phase and 6.6–6.9 km s⁻¹ for the later phase. The apparent S velocities are 4.6 km s⁻¹ for

Table 1 List of the earthquakes exhibiting anomalous later phases at the coastal stations of the Tokai district

Date	Hypocentre (°E)	Hypocentre (°N)	(km)	$\alpha 1$	$\alpha 2$	$\beta 1$	$\beta 2$
				(km s ⁻¹)			
28 December 1980	134.92	33.73	50	7.9	6.6	4.6	3.8
5 March 1981	135.38	33.82	50	—	6.7	—	3.9
2 September 1981	135.53	33.88	50	8.0	6.6	—	3.9
12 November 1981	135.27	33.80	60	8.0	6.6	—	3.9
14 November 1981	134.98	33.65	50	—	6.9	—	3.9
11 February 1982	135.78	34.07	50	8.0	6.7	—	3.8
7 May 1982	135.43	33.92	60	8.1	6.8	—	—
21 June 1982	135.43	33.87	60	8.0	6.6	—	—

$\alpha 1$, $\alpha 2$: apparent P velocities of initial and later phases.
 $\beta 1$, $\beta 2$: apparent S velocities of initial and later phases.

A seismological constraint on the depth of basalt-eclogite transition in a subducting oceanic crust

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The oceanic crust primarily has a basaltic composition^{1,2} which must transform into eclogite on subduction of the oceanic crust beneath continents^{3,4}. There has been a considerable debate about the role of the basalt-eclogite transformation in driving lithospheric plate motion⁵ and in the thermal regime of subducting plates⁶. How important this transformation is in tectonics critically depends on the depth of transformation, which is affected by factors such as temperature^{7,8}, bulk rock chemical composition², water content⁹ and, perhaps, stress environment. Because it would be practically impossible to evaluate all these factors correctly, we attempt here to constrain the transformation depth by a more direct seismological method.

The Philippine Sea plate is now subducting beneath south-western Japan along the Nankai trough at a rate of 4 cm yr⁻¹ to the north-west¹⁰. The lithospheric portion subducted into the mantle is delineated by subcrustal earthquakes in a depth range 30–80 km. They occur in a slab-like zone with a thickness of <10 km that dips at an angle of 10–30° towards north to north-west^{11,12}. The subcrustal seismic zone reaches a depth of 80 km beneath the Kii peninsula and a depth of at least 60 km beneath the Tokai district as shown in Fig. 1. In each of the two regions the seismic zone is concave downwards. The seaward and upward extension of the seismic zone is apparently truncated near the coasts at a depth of ~20 km.

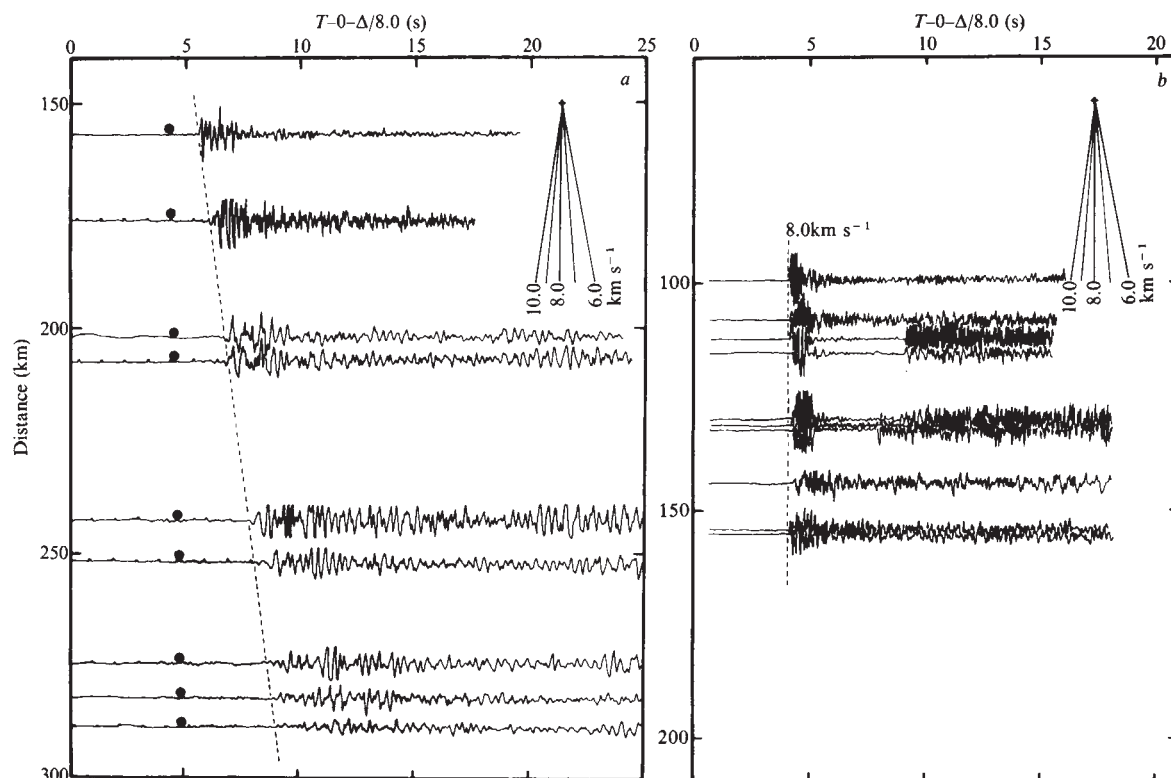


Fig. 2 P-wave seismograms of event A (Fig. 1). *a*, Records at the coastal stations of the Tokai district (filled circles in Fig. 1). ●, Arrival times expected from a standard travel time table. *b*, Records at the inland stations (filled squares in Fig. 1). Amplitudes abruptly diminish a few seconds after the first arrivals because of auto-gain controlling.

the initial phase (based on a single observation) and $3.8\text{--}3.9\text{ km s}^{-1}$ for the later phase. The apparent P and S velocities of the initial phases are somewhat higher than the sub-Moho P and S velocities in this region which are about 7.8 and 4.4 km s^{-1} respectively^{14,15}. The apparent P and S velocities of the later phases are, on the other hand, anomalously low as compared with the sub-Moho P and S velocities.

The P and S later phases of the above kind are not observed at the inland stations. Figure 2*b* shows the P-wave seismograms for event A at stations which are located to the north of the Kii peninsula. In contrast to the seismograms shown in Fig. 2*a*, the first arrivals with an apparent velocity of 8.0 km s^{-1} are very strong and are not followed by appreciable later arrivals.

The later phases are not observed at any stations if earthquake foci are deeper than 60 km . The first arrivals are, instead, clearly identified in the vicinity of their expected arrival times. To establish this observation, we classified the 35 earthquakes into two types by referring to the records at the three coastal stations, which were the only coastal stations before December 1980. If such later arrivals as shown in Fig. 2*a* are observed at all the three stations, the event is categorized into Type I; if not, the event is categorized into Type II. Table 2 shows the result of such classification. If an earthquake is shallower than 60 km , then consistent later arrivals are observed in most cases. If an earthquake is deeper than 60 km , no such later arrivals are observed. The transition of Type I to II, thus occurs at a depth of 60 km .

Figure 3 shows the composite travel time curves in which the initial and the later arrival times read at the coastal stations are plotted as a function of epicentral distance. The plots are made for the events whose focal depths are determined to be 50 km . The apparent velocities of the later P and S phases are about 6.8 and 3.9 km s^{-1} respectively in agreement with the result of Table 1 although the S data scatter rather considerably. As pointed out earlier, these apparent velocities are significantly lower than the sub-Moho velocities. They are rather comparable to the P and S velocities of the lower crust of southwestern Japan^{14,15} and to those of the main layer (Layer 3) of the typical oceanic crust^{2,16}. This approximate coincidence of the observed

Table 2 Classification of subcrustal earthquakes

Depth (km)	No. of earthquakes	
	Type I	Type II
40	2	3
50	17	1
60	4	4
70	0	4
80	0	1

Type I: earthquakes with such later arrivals as shown in Fig. 2*a*.
Type II: earthquakes without such consistent later arrivals.

apparent velocities to the seismic velocities of the lower crust and to those of the oceanic crust leads to the following idea as illustrated in Fig. 4.

In Fig. 4 the Philippine Sea plate, with the oceanic crust at its top, is subducting beneath southwestern Japan at a shallow dip of $10\text{--}20^\circ$ in the Tokai district. The associated oceanic crust is assumed to retain its basaltic mineral assemblage and not to undergo basalt-eclogite transition at least down to a depth of $50\text{--}60\text{ km}$. As the plate progressively proceeds downward and landward away from the Nankai trough, the untransformed oceanic crust comes in direct contact, first, with the bottom of the upper crust, second, with the bottom of the lower crust and, third, with the sub-Moho mantle. There is an increase in seismic velocity from above to below across the first contact zone whereas velocities decrease across the third contact zone: there is little velocity contrast across the second contact zone and coastal seismic stations are located above this second contact zone. Suppose that an earthquake occurs within the untransformed oceanic crust constituting a low-velocity zone in the uppermost mantle. The seismic waves generated are largely trapped in this low-velocity zone and propagate through it as guided waves. They can travel over long distances along the strike of the subducting plate with speeds comparable to the P and S velocities of the untransformed oceanic crust. They suffer less geometric attenuation than ordinary waves and eventually come up to the Earth's surface through the second

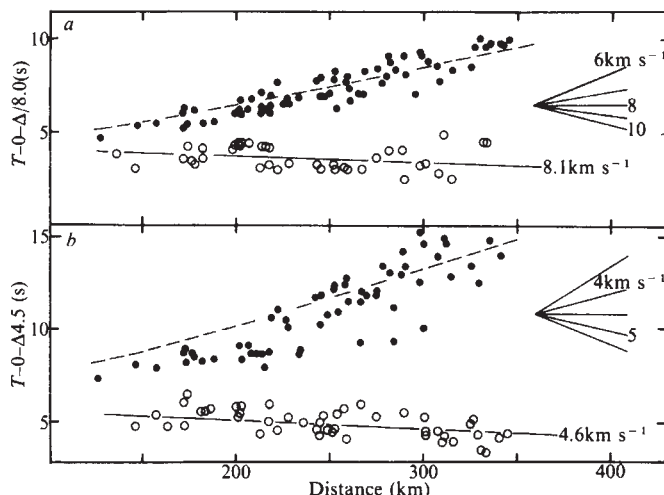


Fig. 3 Travel times versus epicentral distances. ○, ●, Initial and later arrivals respectively. The broken curve indicates a theoretical travel time curve of the waves trapped within the untransformed oceanic crust. *a*, P waves; *b*, S waves.

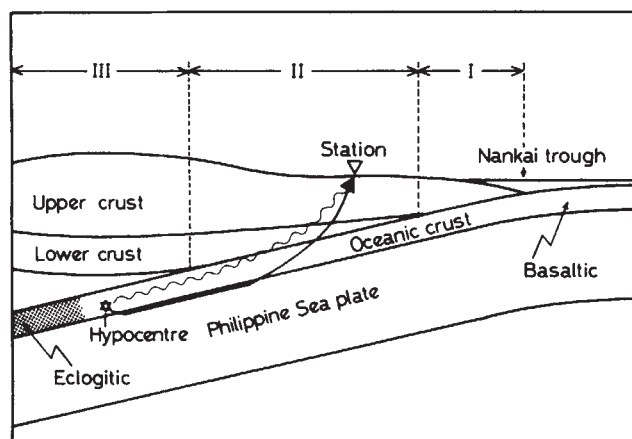


Fig. 4 The Philippine Sea plate subducting beneath southwestern Japan. Ray paths of the first and later arrivals are depicted by a solid curve and a wavy curve respectively. The hypocentre and the station are not in the same cross-section but are far apart along the strike of the Nankai trough. The first, second and third contact zones are denoted by I, II and III.

contact zone to manifest themselves as such distinct later phases as in Fig. 2a. The initial phases perhaps represent the P and S waves propagating as head waves in a high velocity layer of the subducting plate (Fig. 4).

If this interpretation is correct, the travel times of the later phases may be approximated by those of the direct waves which propagate through the sub-Moho mantle with the P and S velocities of the main layer of the oceanic crust ($V_p = 6.8 \text{ km s}^{-1}$, $V_s = 3.9 \text{ km s}^{-1}$). The travel time curves calculated in this way are superposed on the travel time plots of Fig. 3. The agreement between the observation and the calculation is reasonably good for both P and S.

Our model thus explains both the data of apparent velocity and travel time and simultaneously explains why the later phases are observed only at coastal stations. Two immediate conclusions follow: first, the subducting oceanic crust remains untransformed down to a depth of about 60 km; second, subcrustal earthquakes down to this depth must occur within the subducting oceanic crust.

According to the pressure-temperature stability diagram, the main layer of oceanic crust will go into the stability field of eclogite at depths of <20–30 km (ref. 9). However, at such shallow depths the temperature of crustal elements will be <200 °C (ref. 17) and the rate of reaction may be sufficiently slow for the basalt and gabbro to be metastable well into the eclogite stability field. For example, at a temperature of

~400 °C, the basalt-eclogite transition will take place in the geological times of 10^6 – 10^7 yr (ref. 8). At lower temperatures basalt should remain untransformed for extremely long time periods⁸. The Philippine Sea plate is descending into the mantle from the Nankai trough at a vertical rate of ~1 cm yr⁻¹. It takes ~ 5×10^6 yr for the crustal rock of the sea floor to reach a depth of 50–60 km. Our results therefore suggest that the subducting oceanic crust remains untransformed over a period of at least 5×10^6 yr, thus constraining the temperature of the descending lithosphere. Note that the untransformed portion of the subducting crust causes an upward body force in the lithosphere comparable with the downward body force of thermal contraction of the descending lithosphere. With the assumption of a direct transformation from basalt to eclogite, it is very unlikely that the subduction of the Philippine Sea plate along the Nankai trough is driven by the excess weight of the descending slab¹⁸ if its leading edge reaches only a depth of 60–80 km as suggested from the seismicity.

There are two alternative explanations for why distinct later phases are not observed for events deeper than 60 km. First, basalt may transform into eclogite at a depth of 60 km so that the subducting oceanic crust would no longer be a low-velocity zone at greater depths. Table 2 in this case indicates that the transformation from basalt to eclogite is quite sharp. Second, the untransformed oceanic crust may extend further deep below 60 km but earthquakes at greater depths occur outside, perhaps below, it. At the moment, it is difficult to resolve these two alternatives.

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1. Fox, P. J., Schreiber, E. & Peterson, J. J. *J. geophys. Res.* **78**, 5155–5172 (1973).
2. Ringwood, A. E. *Composition and Petrology of the Earth's Mantle*, 51–60 (McGraw-Hill, New York, 1975).
3. Yoder, H. S. & Tilley, C. E. *J. Petrol.* **3**, 342–532 (1962).
4. Green, D. H. & Ringwood, A. E. *J. Geol.* **80**, 277–288 (1972).
5. Ringwood, A. E. *Earth planet. Sci. Lett.* **14**, 233–241 (1972).
6. Delany, J. N. & Helgeson, H. C. *Am. J. Sci.* **278**, 638–686 (1978).
7. Wyllie, P. J. *Bull. geol. Soc. Am.* **93**, 468–476 (1982).
8. Ito, K. & Kennedy, G. C. *Geophys. Monogr. No. 14*, 303–314, (1971).
9. Ahrens, T. J. & Schubert, G. S. *Rev. Geophys. Space Phys.* **13**, 383–400 (1975).
10. Seno, T. *Tectonophysics* **42**, 209–226 (1977).
11. Mizoue, M. *Proc. Symp. Earthquake Prediction Research*, 97–105 (1977) (in Japanese).
12. Ukiwa, M. *Tectonics* **1**, 543–571 (1982).
13. Mizoue, M. *Bull. Earthq. Res. Inst.* **49**, 33–62 (1971).
14. Aoki, H. et al. *J. Phys. Earth* **20**, 197–233 (1972).
15. Ukawa, M. & Fukao, Y. *Tectonophysics* **77**, 233–256 (1981).
16. Murauchi, S. et al. *J. geophys. Res.* **73**, 3143–3171 (1968).
17. Turcotte, D. L. & Schubert, G. S. *J. geophys. Res.* **78**, 5876–5886 (1973).
18. Forsyth, D. W. & Uyeda, S. *Geophys. J. R. astr. Soc.* **43**, 163–200 (1975).

Two new pre-trilobite faunas from western North America

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Perhaps the greatest puzzle in the history of life is the nature and cause of events comprising the Precambrian-Cambrian evolutionary radiation of Metazoa. The major stumbling blocks to resolving this puzzle are a scarcity of late Precambrian and earliest Cambrian faunas in the fossil record and difficulties in correlating the few faunas that are known. The most diverse and best-known basal Cambrian (Tommotian) faunas are from Siberia^{1–3}, and other pre-trilobite shelly faunas are known from the UK^{4,5}, Newfoundland^{6,7}, Mongolia⁸, China^{9–11}, Scandinavia¹² and northwestern Canada¹³. Here we report the discovery of two new early Cambrian shelly faunas from western

North America, one from Nevada and the other from Sonora, Mexico. These faunas consist of small calcitic tubes and cones, some of which are closely related to previously known fossils from other areas. Both faunas are found hundreds of metres below the first trilobite occurrences, below the first presumed trilobite trace fossils, and seem to be earliest Cambrian in age. This is the first discovery of a diverse pre-trilobite shelly fauna in the classic Precambrian–Cambrian sequence of western Nevada and the White-Inyo Mountains of California, and the first report of pre-trilobite small shelly fossils from Mexico. These new finds may lead to an understanding of the pattern and dynamics of the Precambrian–Cambrian metazoan radiation, and to improved biostratigraphical correlations of the strata recording that event.

For almost a decade, geologists working in well-exposed late Precambrian and early Cambrian sections on Mt Dunfee, near Gold Point, Nevada (Fig. 1), have observed small tubular to conical fossils in the Lower Deep Spring Formation (Fig. 2a). While identified in the field as *Wyattia*, an enigmatic conical fossil known only from the Reed Dolomite in the White-Inyo Mountains of California (Fig. 2a)^{14,15}, these fossils have never been studied. When bulk rock samples from the Lower Deep Spring Formation were dissolved in dilute (10%) acetic acid, in an attempt to free the fossils from the limestone matrix, it was discovered that *Wyattia* was not present. Instead, it was found that an abundant fauna composed of at least four elements had been mistaken for *Wyattia*. These fossils are poorly preserved tubular to conical calcitic fossils, three of which belong to genera known from other regions. X-ray analysis shows no significant silica, magnesium or phosphate present in the fossils. It remains uncertain why the fossils etch out of the rock.

The small (1 mm diameter), distinctive tube *Coleoloides* is the least common element in the Mt Dunfee fauna; only a single specimen has been recovered (Fig. 3a). *Coleoloides* is known from the Lower Cambrian of Newfoundland^{6,7,16}, eastern Massachusetts⁶, the UK^{4,5}, Scandinavia¹² and Siberia¹. In each area, except Massachusetts, *Coleoloides* is a major component of the pre-trilobite (equivalent to the non-trilobite of ref. 17) shelly fauna. In Massachusetts, *Coleoloides* occurs with the trilobite *Strenuella strenua*^{18–20}. *Coleoloides* also occurs with early trilobites in the UK^{17,21} and possibly Bornholm²². Thus, the range of this genus seems to extend from Tommotian to Atdabanian time-equivalent rocks (see ref. 23 for a tentative correlation of Lower Cambrian strata). Far more common at Mt Dunfee is a second small coleolid, *Coleolella* sp. (Fig. 3b), otherwise known from a single species occurring in Tommotian strata of the Aldan River region of Siberia and from Maidiping, China^{1,24}. *Coleolella* sp. also attains a diameter of ~1 mm. The phylogenetic affinities of the Coleolidae are uncertain. Some authors have suggested an annelid affinity^{1,25}, others molluscan²⁶ or pogonophoran affinities²⁷.

Also present in the Mt Dunfee fauna is the hyolith *Salanytheca* sp. (Fig. 3c), described from the Tommotian strata of Siberia and Mongolia⁸. This fossil is larger than the coleolids and commonly attains diameters up to 5 mm. The fourth element is a rare, peculiar cone with an irregular, wrinkled surface (Fig. 3d). The wrinkled cone is the same size as the *Salanytheca* sp. and its affinities are unknown. A fifth fossil having a circular cross-section (3 mm diameter) and widely spaced longitudinal ribs also occurs in the fauna but cannot be adequately described from the available material. Residues from the acid dissolutions have failed to yield any protoconodonts or other phosphatic fossils.

The Mt Dunfee fauna occurs ~1,000 m below the first trilobites and 400 m below the first trace fossils that can be ascribed to trilobite activities²⁸. The only other shelly fossil of comparable age described from this region is *Wyattia*¹⁴, which occurs in the underlying Reed Dolomite in the White-Inyo Mountains of eastern California. The boundary between the Upper Reed Dolomite and the Lower Deep Spring is diagenetic and probably time-transgressive. Hence, *Wyattia* and the Mt

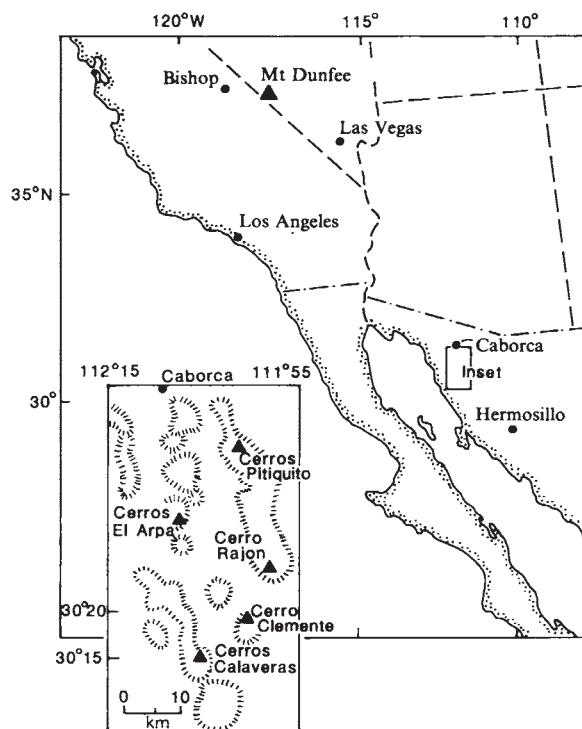


Fig. 1 Generalized map showing the locations of the sites in Nevada and Sonora where the faunas were collected. ▲, Fossil locality.

Dunfee fauna are possibly coeval and might not differ greatly in age.

A second fauna was discovered in the Cerros Pitiquito, Cerro Rajón, Cerro Clemente, and Cerros Calaveras, south of Caborca, Mexico (Fig. 1). These fossils were found in a sandy dolomitic limestone, part of an unnamed unit below the Puerto Blanco Formation (Fig. 2b). The fossils occur 445 m below a Puerto Blanco limestone bed containing brachiopods (probably belonging to the *Fallotaspis* Zone) and 535 m below the oldest known Puerto Blanco Formation trilobite. Like the Mt Dunfee fauna, the Caborca fauna is calcitic but in some ranges the fossils are weakly silicified. Excellent specimens can be collected on well-weathered surfaces.

The Caborca fauna consists of four tubular morphologies and a single conical morph. Smooth-surfaced, multiple-walled tubes that average 2–3 mm in diameter are common. The fossil in Fig. 3e has not yet been sectioned, but may consist of several nested tubular or conical shells (common for hyoliths). Also common is a smaller (1–2 mm diameter) smooth-surfaced, single-walled tube (Fig. 3f). This second tube resembles some coleolids known from the USSR but the fragmentary material available precludes definitive taxonomic assignment. Larger (0.5–3.5 mm diameter), irregularly annulated, single-walled tapering tubes constitute the third morphology (Fig. 3g) and are similar to the wrinkled cones of the Mt Dunfee fauna, but do not taper as strongly. The fourth tubular fossil is a regularly annulated tube represented by only a single specimen (1.7 mm diameter) (Fig. 3h). This shell fragment, broken on both ends, is superficially similar to the weakly tapering hyolith *Turcutheca* although it lacks growth lines which curve towards the apex, a characteristic of this genus. A smooth-walled conical fossil (maximum diameter 2.0 mm, length 5.4 mm) also occurs in this fauna.

The two faunas reported here are unusual with respect to many other early Cambrian faunas due to their lack of phosphatic elements. Unlike more modern faunas, pre-trilobite faunas are relatively rich in phosphatic fossils such as hyolithelids, *Mobergella*, *Lapworthella*, *Tommotia* and inarticulate brachiopods^{23,29,30}. The lack of phosphatic fossils at Mt Dunfee and Caborca could be due as much to diagenetic as to evolution-

Fig. 2 Stratigraphical placement of faunas from Mt Dunfee and Caborca. *a*, Stratigraphical sequence of late Precambrian and early Cambrian rocks of Esmeralda County, Nevada, showing the horizon where the fauna occurs. The occurrences of the shelly faunas are indicated by a cone and the first trilobites in each section by a trilobite symbol. *b*, Stratigraphical sequence of late Precambrian and early Cambrian strata in Sonora, Mexico. The position of the Precambrian–Cambrian boundary is uncertain in both areas. Stratigraphical equivalence of the two sections is not implied. U, Upper; M, Middle; L, Lower.

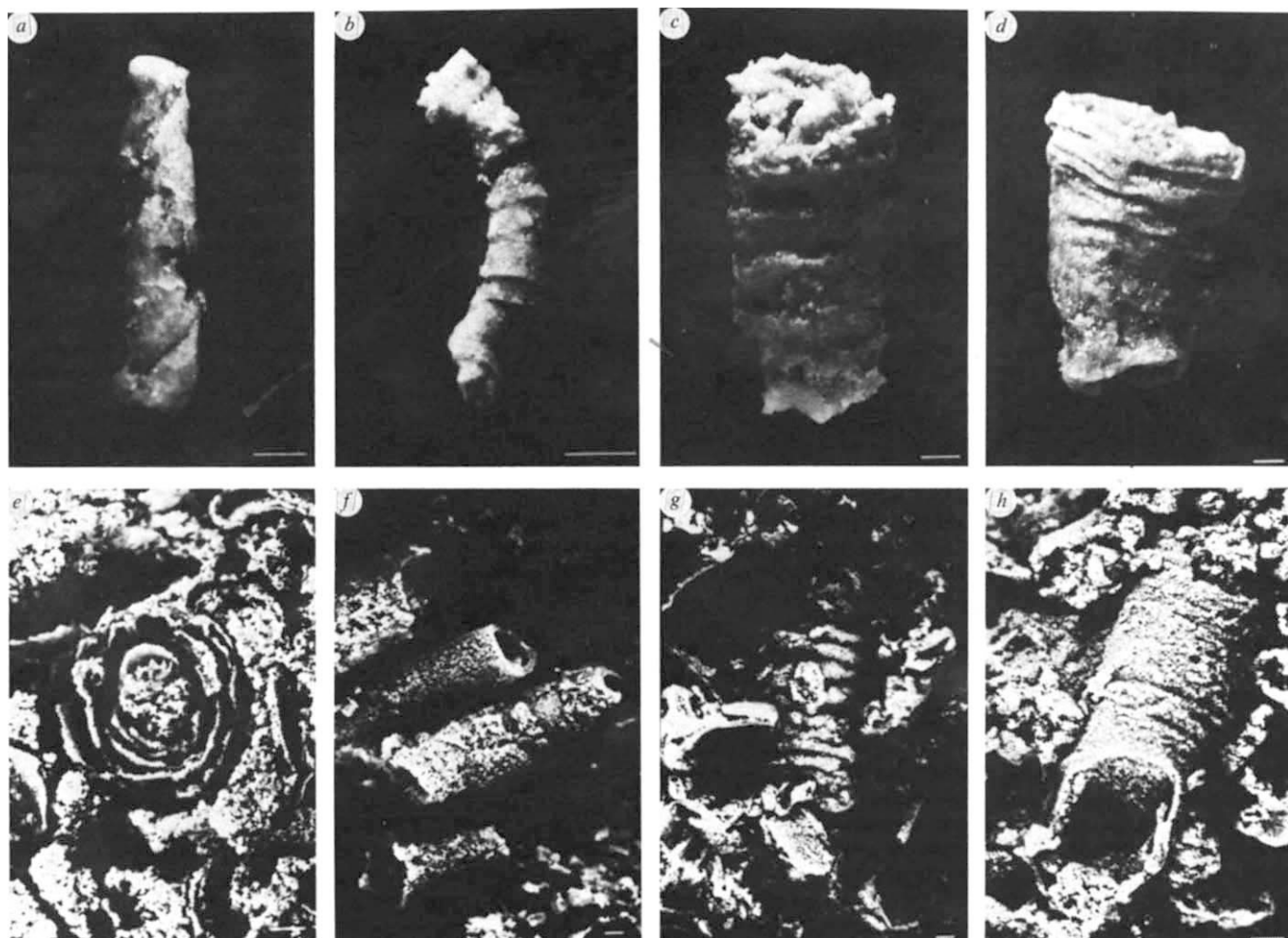
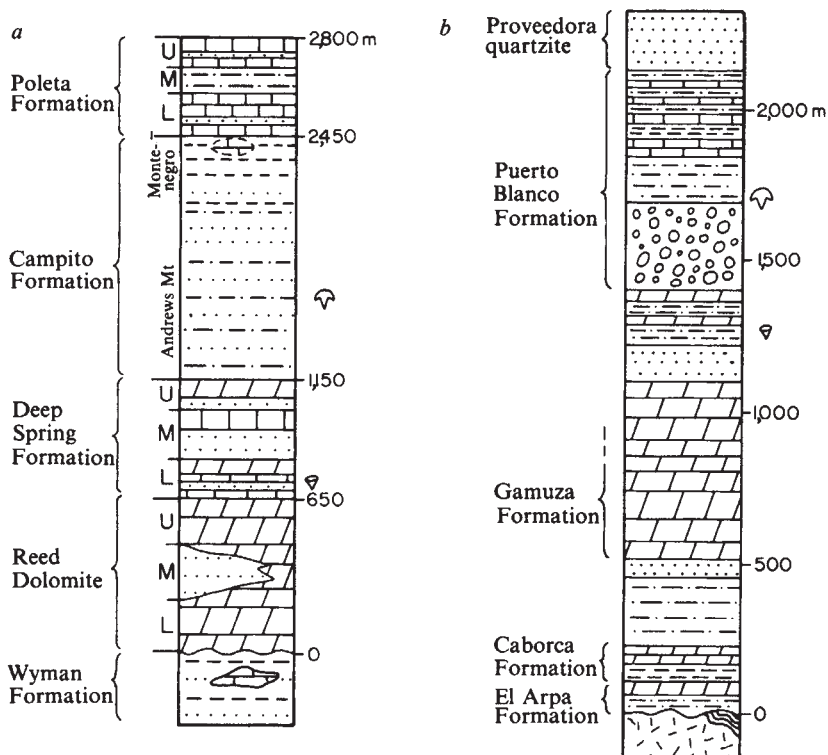


Fig. 3 Pre-trilobite fossils from Mt Dunfee (*a–d*) and Caborca (*e–h*). *a*, *Coleoloides* sp. Note the longitudinal ribs and furrow on the bottom quarter of the fossil that spiral behind and reappear towards the top of the photograph. *b*, *Coleolella* sp. *c*, *Salanythea* sp. This fossil differs from *B* in taper, its larger size and the different shape and spacing of the ribs. *d*, Undescribed fossil. *e*, Multiple-walled tubular fossil from the Cerro Rajón. *f*, Smooth, single-walled shell from the Cerro Rajón. *g*, Irregularly annulated tube from the Cerro Rajón. *h*, Regularly annulated tube. Scale bars, 1 mm.

ary or palaeobiogeographical factors. Both stratigraphical sections have been exposed to low-grade metamorphism and the fossils at both localities are extremely recrystallized. X-ray analyses of specimens from Mt Dunfee and electron microprobe analysis of Caborca specimens indicate that no phosphate is present although phosphatic fossils could have been a part of the original faunas. Alternatively, the lack of phosphatic fossils might be related to the sequence of facies represented in western North America, as suggested elsewhere for other faunas²³.

Based on stratigraphical position and taxonomic similarities, the Mt Dunfee and Caborca faunas seem to represent the approximate time-equivalents of the basal Cambrian Tommotian Stage of Siberia and Meishucun Stages of China, and the pre-trilobite shelly faunas of Europe and Avalonia. The

occurrence of these faunas matches the pattern observed elsewhere of small, shelly fossils preceding the first trilobites²³, and supports the idea that a basal Cambrian stage based on pre-trilobite shelly fossils can be recognized and correlated throughout the world. Because *Wyattia* has not been found outside the White-Inyo Mountains, the Mt Dunfee and Caborca faunas provide the first opportunity for pre-trilobite biostratigraphical correlations between the southern Great Basin and other parts of the world. These faunas also allow the first glimpse of the evolutionary sequence of pre-trilobite shelly metazoans in western North America; further studies should evaluate this in greater detail.

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1. Rozanov, A. Yu. et al. (ed. Raaben, M. E.) *The Tommotian Stage and the Cambrian Lower Boundary Problem* (Amerind, 1981).
2. Zhuravleva, I. T. *Am. J. Sci.* **269**, 417–445 (1970).
3. Matthews, S. C. & Missarzhevsky, V. V. *J. geol. Soc. Lond.* **131**, 289–304 (1975).
4. Cobbold, E. S. *Geol. Mag.* **6**, 149–158 (1919).
5. Brasier, M. D., Hewitt, R. A. & Brasier, C. J. *Geol. Mag.* **115**, 21–36 (1978).
6. Matthew, G. F. *Proc. Trans. R. Soc. Can.* **5**, 97–119 (1899).
7. Hutchinson, R. D. *Bull. geol. Surv. Can.* **88**, 1–156 (1962).
8. Missarzhevsky, V. V. *Palaeont. J.* **1980**, 18–25 (1980).
9. Jiang, Z. *Bull. Chin. Acad. geol. Sci.* **1**, 2, 75–92 (1980).
10. Jiang, Z. *U.S. geol. Surv. Open File Rep.* 81–743, 98–99 (1981).
11. Lu, Y. & Zhu, Z. *U.S. geol. Surv. Open File Rep.* 81–743, 121–122 (1982).
12. Poulsen, C. *Meddr danske Vidensk. Selsk. mat.-fys.* **36** (1967).
13. Conway Morris, S. & Fritz, W. H. *Nature* **286**, 381–384 (1980).
14. Taylor, M. E. *Science* **153**, 198–201 (1966).
15. Palmer, A. R. in *Cambrian of the New World* 1–79 (Wiley-Interscience, Chichester and New York, 1971).

16. Bengtson, S. & Fletcher, R. P. *U.S. geol. Surv. Open File Rep.* 81–743, 18 (1981).
17. Brasier, M. D. & Hewitt, R. A. *U.S. geol. Surv. Open File Rep.* 81–743, 29–33 (1981).
18. Shaler, N. S. & Foerste, A. F. *Harvard Coll. Mus. comp. Zool.* **16**, 13–41 (1888).
19. Shaw, A. B. *J. Palaeont.* **24**, 577–590 (1950).
20. Palmer, A. R. in *Cambrian of the New World* 170–217 (Wiley-Interscience, Chichester and New York, 1971).
21. Rushton, A. W. A. in *Cambrian of the British Isles, Norden and Spitzbergen* 43–121 (Wiley-Interscience, Chichester and New York, 1974).
22. Poulsen, V. *Geol. Mag.* **115**, 131–136 (1978).
23. Brasier, M. D. in *The Origin of Major Invertebrate Groups* 103–159 (Academic, London and New York, 1979).
24. Xing, Y., Ding, Q. & Luo, H. *Precambrian Res.* **17**, 77–85 (1982).
25. Glaessner, M. F. *J. geol. Soc. Lond.* **132**, 259–275 (1976).
26. Fisher, D. W. in *Treatise on Invertebrate Paleontology Pt C*, W98–W143 (Univ. Kansas Press, Lawrence, 1962).
27. Brasier, M. D. & Hewitt, R. A. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **27**, 35–57 (1979).
28. Alpert, S. P. in *Trace Fossils and the Basal Cambrian Boundary* 1–8 (Seel House, 1977).
29. Stanley, S. M. *Am. J. Sci.* **276**, 56–76 (1976).
30. Durham, J. W. A. *Rev. Earth planet. Sci.* **6**, 21–42 (1978).

Aspartic acid racemization in narwhal teeth

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Sometimes referred to as sea unicorns, narwhals (*Monodon monoceros*) are Arctic cetaceans which throughout history have been revered as unique and mystical animals. In pre-nineteenth century societies, the long, up to 2 m in length, counterclockwise spiralling tooth of the male narwhal was thought to be the 'tusk' or alicorn of the legendary unicorn^{1,2}. Alicorns were also thought^{1,2} to possess a variety of powers such as being able to detect and prevent poisoning. During the fifteenth and sixteenth centuries, narwhal tusks had a value of ~10 times their weight in gold³, and thus they were often used to settle debts. Extensive hunting by seventeenth–twentieth century European and American whalers probably caused short-term reductions, but apparently no long-term effects, in the narwhal population⁴. Only Eskimos continue to hunt narwhals mainly for their tusks, although they also consider the skin (*muktuk*) a delicacy³. Many recent studies of narwhals have been concerned with their distribution, abundance, life cycle, and behaviour^{4–11} in the hope of aiding the management of this exploited species. Yet little is known about the population dynamics of narwhals, because determining the age of any particular animal is difficult. We report here that the extent of aspartic acid racemization in narwhal teeth provides a means for determining the age of narwhals. This method may be particularly useful for mature females, the ages of which have previously been difficult or impossible to determine.

The 'tusk' of the male narwhal is actually a maxillary tooth which erupts through the left side of the upper jaw during the first year of life^{2,5–11}. A series of fairly regularly spaced dentinal growth layer groups (GLG) are present in the tusk which indicates that this exposed tooth apparently continues to grow throughout the animal's lifetime. The number of GLGs in the tusk provide a potential means of ageing male narwhals¹². Although the GLG layer deposition rate in the male narwhal tusk is not known with any certainty, it is thought to be similar to that of the teeth of other cetaceans and have a value of ~1 GLG yr⁻¹ (refs 13–15). Because females do not (usually) have tusks their ages are more difficult to evaluate than males¹⁶.

Males also have an unerupted maxillary tooth which initially develops simultaneously with the exposed one but does not erupt although there are rare instances¹⁷ where both teeth erupt. In females both maxillary teeth generally remain unerupted but occasionally females¹⁷ also have a tusk. Eventually, the pulp cavity of the embedded or unerupted tooth becomes occluded, and it stops growing. Although GLGs are present in the unerupted maxillary teeth, their correlation with the animal's age is difficult to interpret, especially in mature animals where the teeth are occluded¹⁶.

Due to the lack of a routine procedure for evaluating the ages of both male and female narwhals, the amino acid racemization technique^{18–20} was investigated. Since narwhals live only in the Arctic, the male's exposed tusk has been maintained at temperatures near the freezing point of seawater and, at 0 °C, the estimated half-life for aspartic acid racemization in teeth is ~2 Myr (calculated from the equation given in ref. 21). Thus, there should be no detectable racemization in the male's tusk during the animal's lifetime. The embedded teeth, however, have been maintained at ~37 °C, that is, mammalian body temperature. If narwhals have a longevity similar to that of other mammalian species, detectable racemization should therefore be observable in the tip of the unerupted teeth^{18–20}.

Narwhal teeth were collected in the summer of 1978 during an Inuit ice edge hunt at Sedleoorosuk, ~45 km east of Pond Inlet, Northern Baffin Island, North-west Territories, Canada. Teeth from two females (1423 and 1424; body length 4.04 and 3.70 m, respectively) and two males (1443 and 1444; body

length 4.52 and 4.36 m, respectively) were collected from kills made by Eskimo hunters. The large erupted tusks of the males were longitudinally sectioned using a diamond saw. One surface was longitudinally sanded and visually examined for GLG counts, then acid (phosphoric) etched, washed in water and dried with a light wipe of acetone. The dried surface was then rubbed with a flat lead carpenter's pencil. Visual counts of the number of GLGs of the etched and pencil-rubbed surfaces were then made by two or more observers; the definition of a GLG was based on the most recent consensus¹².

Embedded teeth were dissected out of the skull, cast into fibreglass resin and then bisected longitudinally with a diamond saw. The cut surface was then sanded, etched and GLG counts determined using the procedure already described for the erupted tusk.

Samples for racemization analyses were removed from the cut and etched surfaces with a cast saw in a bath of running water to avoid heating. Samples were chosen to comprise the hard tissue deposited within a single GLG section at various places in one erupted tusk, and at the tips of the embedded teeth of all four animals. Small (~0.1 g) pieces of the various narwhal teeth were hydrolysed in 6 M HCl for 6 h and then processed and analysed for the extent of aspartic acid racemization (D/L Asp ratios) using the procedures described elsewhere^{18,20,22,23}. The aspartic acid racemization results for the various samples are given in Table 1.

As expected, no systematic change in the extent of aspartic acid racemization was detected in the exposed tusk. The D/L Asp ratio at the base (or butt end) of the tusk was the same as that at the tip and the average D/L Asp ratio (for example, 0.019 ± 0.006) was close to the $t = 0$ D/L Asp ratio (for example, 0.014) determined for human teeth¹⁸. The small extent of aspartic acid racemization which was determined in the tusk arises during the acid hydrolysis step used in sample processing²⁴.

In contrast to the results from the exposed tusk, detectable racemization of aspartic acid could be measured in the samples taken from the tip of the unerupted teeth and thus these results can be used to estimate the age of the various animals¹⁸⁻²⁰. Two of the unerupted teeth came from males which enables us to calculate, based on the ages derived from aspartic acid racemization, the GLG deposition rate in the erupted male tusks (Table 2). The average GLG deposition rate calculated from the aspartic acid racemization derived ages is $0.73 (\pm 0.15)$ GLG yr⁻¹. This is somewhat less than the value of ~1 GLG yr⁻¹ estimated for the teeth of some other cetaceans¹³⁻¹⁵. [It is possible, considering the uncertainties of the estimates that the two values are statistically equivalent, however.] The slower GLG deposition rate calculated from aspartic acid racemization might be attributable to wear at the tip of the tusk which would result in the loss of some growth layers.

Table 1 The extent of aspartic acid racemization in various narwhal teeth

Sample no. and description	D/L Asp
Erupted or exposed tooth (tusk)	
1444♂ 4 cm*	0.017
26.5 cm	0.028
67 cm	0.011
178 cm	0.021
206.5 cm	0.017
Average = 0.019 ± 0.006	
Unerupted teeth	
1444♂ Prenatal layer at tip	0.060, 0.063†
fifth GLG from tip	0.058, 0.056†
1443♂ Near tip	0.048, 0.048†
1423♀ Near tip	0.058, 0.059†
1424♀ Near tip	0.040, 0.039†

* Distance from the base or butt end of the tusk. The base of the tusk represents the layer deposited in 1978. 37 GLGs were counted in this tusk.

† Duplicate analysis.

Table 2 Growth layer group (GLG) deposition rate in male narwhal tusks calculated from aspartic acid racemization derived ages

Sample no.	Total no.* of GLGs in erupted tusk	Animal's age from aspartic acid racemization† (yr)	GLG deposition rate (GLG yr ⁻¹)
1444	37 ~32‡	52 (± ~7) 46 (± ~7)	0.7 (± 0.15) 0.7 (± 0.15)
1443	29	36 (± ~5)	0.8 (± 0.15)

* The estimated error in reading total GLGs is ± 5–10%.

† Calculated from D/L asp ratios given in Table 1 for the unerupted teeth and the equation [taken from ref. 18],

$$\text{Age (yr)} = \frac{\ln(1 + D/L) - \ln(1 + D/L)_{t=0}}{7.87(\pm 0.39) \times 10^{-4}}$$

The average D/L asp ratio in the exposed tusk (see Table 1) was used for the $t = 0$ term. The estimated uncertainties in the ages are the total accumulative error which arises from uncertainties in the measured D/L asp ratios and the rate constant for interconversion of the L- and D- aspartic acid enantiomers.

‡ The racemization analyses were carried out on the fifth GLG section of the unerupted tusk (see Table 1), which corresponds to ~32 GLGs in the exposed tusk.

However, inspection of the tip of the tusks indicated that the neonatal line is present in 1443 which suggests the GLG counts for this sample are complete and for 1444, although the neonatal line is not clearly visible, the tip of the tusk was not obviously worn so we assume that few if any GLGs are missing. Because the racemization derived rate is actually a value averaged over the animal's age, the slower rate calculated from racemization may indicate that GLG deposition slows with increasing narwhal age. Differences in body temperature between narwhals and humans (the aspartic acid ages are based on the analyses of known age human teeth¹⁸) cannot explain the slower GLG deposition rate derived from racemization as this would require that the body temperature of narwhals be ~2 °C higher than that of humans, which seems unreasonable.

These investigations indicate that the extent of aspartic acid racemization in the tip of the unerupted tooth of narwhals can be used to obtain age estimates for these animals. This method when used in conjunction with the ages obtained from GLG counts should provide important information about the age structure of narwhal populations and should be particularly useful for age determinations of mature females. For example, the estimated ages of the two females in Table 1 are 48 (± 6) yr for 1423 and 25 (± 3) yr for 1424; it was not possible to evaluate the age of these two animals using other methods¹².

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- Shepard, O. *The Lore of the Unicorn* (Barnes and Noble, New York, 1967).
- Arvy, L. *Acta zool. Path. Antverp.* **73**, 43–118 (1978).
- Reeves, R. *Defenders* **51**, 222–227 (1976).
- Mitchell, E. & Reeves, R. R. *Rep. int. Whaling Comm.* **31**, 645–682 (1981).
- Newman, M. A. in *Marine Mammals of Eastern North Pacific and Arctic Waters* (ed. Haley, D.) 138–144 (Pacific Search Press, Seattle, Washington, 1978).
- Davis, R. A., Richardson, W. J., Johnson, S. R. & Renaud, W. E. *Rep. int. Whaling Comm.* **28**, 209–215 (1978).
- Mansfield, A. A., Smith, T. G. & Beck, B. J. *Fish. Res. Bd. Canada* **32**, 1041–1046 (1975).
- Finley, K. J., Davis, R. A. & Silverman, H. B. *Rep. int. Whaling Comm.* **30**, 459–464 (1980).
- Silverman, H. B. & Dunbar, M. J. *Nature* **284**, 57–58 (1980).
- Best, R. C. *Can. J. Zool.* **59**, 2386–2393 (1981).
- Finley, K. J. & Miller, G. W. *Rep. int. Whaling Comm.* **32**, 449–460 (1982).
- Perrin, W. F. & Myrick, A. C. (eds) *Rep. int. Whaling Comm. Spec. Iss.* **3**, 1–50 (1980).
- Gambell, R. *Discovery Rep.* **35**, 199–358 (1972).
- Best, P. B. S. *Afr. J. Sci.* **72**, 216–218 (1976).

15. Gurevich, V. S., Stewart, B. S. & Cornell, L. H. *Rep. int. Whaling Comm. Spec. Iss.* 3, 165-169 (1980).
16. Hay, K. A. *Rep. int. Whaling Comm. Spec. Iss.* 3, 119-132 (1980).
17. Clark, J. W. *Proc. zool. Soc. Lond.* 1871, 42-58 (1871).
18. Helfman, P. M. & Bada, J. L. *Nature* 262, 279-281 (1976).
19. Bada, J. L. & Brown, S. *Trends biochem. Sci.* 5, III-V (1980).
20. Bada, J. L., Brown, S. & Masters, P. M. *Rep. int. Whaling Comm. Spec. Iss.* 3, 113-118 (1980).
21. Bada, J. L. *Earth planet. Sci. Lett.* 55, 292-298 (1981).
22. Masters, P. M., Bada, J. L. *Adv. Chem. Ser. No.* 171, 117-138 (1978).
23. Masters, P. M. *Calcif. Tissue Int.* 35, 43-47 (1983).
24. Liardon, R. & Jost, R. *Int. J. Peptide Protein Res.* 18, 500-505 (1981).

Cerebral brain endocast pattern of *Australopithecus afarensis* hominid

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It is rare for cerebral convolutional details to be imprinted on the internal table of cranial bone of fossil hominids. To date, the South African examples have shown the best detail, although surrounded by considerable controversy¹⁻³. Given that the Hadar specimens are the oldest hominids known (3-4 Myr BP)^{4,5}, and have been the subject of considerable debate and interpretation, it is fortunate that such details are found on the cranial bones of at least one specimen, AL 162-28, a small adult⁶. I report here a preliminary description of that endocast and it appears that despite its smallish pongid-sized brain, some degree of cerebral organization had occurred almost 3-4 Myr ago towards a more human pattern. If correct, this would mean that brain size increase may well have followed locomotion, but that brain organization may have occurred early in hominid evolution. Such a finding would call for a re-examination of our understanding about human evolution, and would require much more caution in assuming either 'gradualist' or 'punctuated equilibria' models in hominid evolution^{7,8}.

The AL 162-28 endocast was made at the Cleveland Museum of Natural History on the original hominid fragment with the assistance of Mr Bruce Lattimer and Mr Bill Kimble, using Admold rubber latex, a technique described elsewhere⁹. The endocast represents a small portion of both left and right posterior parietal fragments, and a partial squamal portion of the occipital bone. It measures approximately 82 mm left to right, and 70 mm posterior to anterior. The measurements suggest a volume of just less than 400 ml, based on comparisons with the Taung endocast, which is 404 ml.

Figure 1 is a line-stipple drawing from a dorsal-posterior view, provided by Mr John Gurche. This provides more detail than a black and white photograph. Of particular interest is the furrow A, which is relatively straight, in an anterior-posterior orientation and approximately 20 mm in length. The essential question to be answered is as follows: is this sulcus (1) the lateral calcarine, (2) the interparietal, or (3) an unusual, unnamed pattern not present in extant pongids or hominids? The first possibility would suggest the retention of a *Pan* pattern in early hominids. However, if it were considered to be the interparietal sulcus, a clear departure from a *Pan* cerebral pattern in the distribution of parietal and occipital cerebral cortex would be the case. The last possibility would indicate that the Hadar cerebral morphology was not a retention of a *Pan* character, and would raise considerable questions about the supposed split between the African pongids and early hominids. I consider the second possibility, that is, an early human-like pattern, most appropriate.

Despite well-known variability in the convolutional detail of higher primates, particularly in tertiary gyri and sulci^{10,11}, the genus *Pan* does show certain invariant morphological characteristics which can be briefly listed as follows: (1) there is always a lunate sulcus (*affenspalte*) which provides the approximate anterior boundary for primary visual striate cortex; (2) the lateral calcarine always begins approximate to the occipital

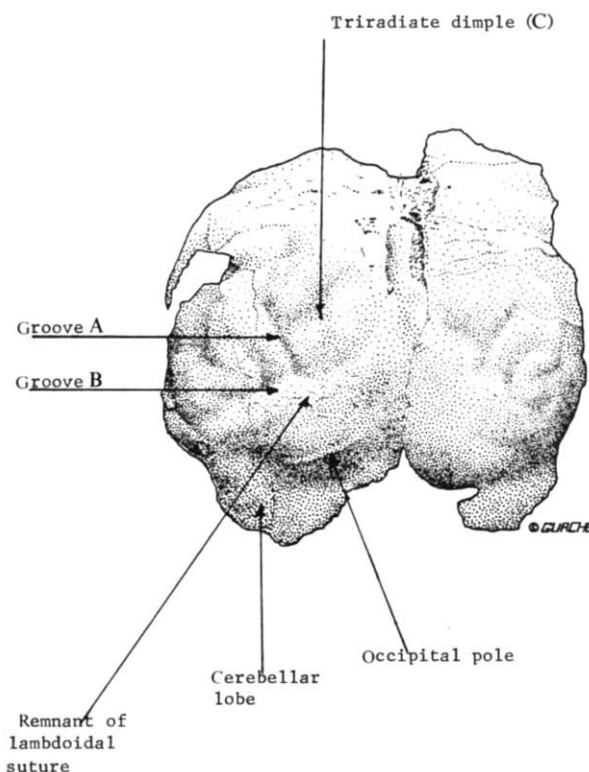


Fig. 1. A posterior and dorsal view showing the available cerebral detail on the Hadar AL 162-28 endocast. Groove A is the sulcus under consideration as to whether it is the lateral calcarine or interparietal. Groove B, at the posterior end of groove A, is not a cerebral sulcus, but a depression caused by the inferior lip of the posterior portion of the parietal bone at the lambdoid suture. C indicates the small triradiate depression well medial to groove A. Note the lack of any convex-anterior sulcus at the anterior limit of groove A which could be a *Pan* lunate sulcus. In comparison with Fig. 2, this arrangement does not suggest a *Pan* morphological pattern. Note also that there is no strong indication of the straight or transverse sinus between the occipital and cerebellar lobes.

pole, courses antero-lateral, and always terminates before the lunate sulcus; (3) the interparietal sulcus, separating superior and inferior parietal lobules, is always anterior to the lunate sulcus, abuts the lunate posteriorly and courses anterior to the postcentral gyrus, where it terminates. The course of the interparietal varies in curvature from almost straight to tortuous, but the invariances described are always there¹²⁻¹⁴. (The same may be said for *Gorilla*, *Pongo* and *Hylobates*; see Fig. 2.) Does the Hadar AL 162-28 fragment follow the above invariant *Pan* pattern? (As the Hadar material is African, *Pan* is the most appropriate comparison.)

Table 1 provides some metric (mm) observations on five *Pan troglodytes* brain (not endo-) casts (Fig. 2), and the AL 162-28 specimen. While I have taken issue with linear chord and arc measurements on endocasts as a technique to demonstrate evolutionary trends, no other techniques are applicable at the present time, including stereoplotting¹⁵. Two outstanding measurements appear for the AL 162-28 specimen: in Table 1a, the distance from the midsagittal plane to the most lateral extension of the lateral calcarine fissure is 22 mm for the Hadar specimen, which is well below the 27 mm value for a smaller 330 ml chimpanzee brain cast. This strongly suggests that groove A in Fig. 1 is not the lateral calcarine. Secondly, Table 1e, the distance of the occipital pole to the anterior end of the interparietal is only 29 mm on the Hadar specimen, conspicuously lower than the values for smaller-brained chimpanzees. This strongly suggests that the groove A is not extended posterior-anterior as in *Pan*, but that its posterior end is more distally located. The distance of the midsagittal plane to the most lateral extension of the interparietal sulcus at the lunate

Table 1 Measurements of *Pan troglodytes* brain casts and the Hadar AL 162-28 endocast

Measurement (mm)			AL 162-28 endocast	1	2	3	4	5
a	Midsagittal plane to lateral end of lateral calcarine	C	22	27	29	32	36	33
		A	23-24	29	33	35	40	36
b	Occipital pole to anterior end of lateral calcarine	C	29	30	29	29	32	29
		A	29-30	30	30	30	34	30
c	Midsagittal plane to lateral extension of interparietal sulcus at junction with lunate sulcus	C	15	21	12-13	16	18	19
		A	15	21	12-13	16	18	19
d	Midsagittal plane to lateral extension of interparietal at anterior end	C	22	24	22	26	29	27
		A	22	24	22	26	29	27
e	Occipital pole to anterior end of interparietal sulcus	C	29	49	51	50	51	54
		A	29-30	54	58	55	52	58
f	Estimated volume (ml)		375-400	330	245	280	290	414

Both chord (C) and arc (A) measurements (mm) are given for the AL 162-28 Hadar endocast, and five chimpanzee brain casts from my collection. The measurements were taken by flexible steel tape, and sliding calipers. In column 1 the measurements for the endocast, based on the chimpanzee brain morphology, are given. The groove *a* under consideration in the Hadar hominid specimen is treated as if it were the corresponding landmark. For example in row 1, the groove A of AL 162-28 is treated as if it were the lateral calcarine sulcus.

(Table 1c) is compatible with considering groove A on the Hadar fragment as interparietal. In Table 1b, groove A is assumed to be the lateral calcarine remnant on the measurement, 29 mm, this is compatible (this value for the Hadar specimen is quite low, even when compared with the *Pan troglodytes* brain cast number 3) with an estimated volume of 280 ml. These measurements strongly suggest that the cerebral morphology of the Hadar specimen is hominid and not pongid in its pattern, and that groove A in Fig. 1 is more correctly identified as the interparietal sulcus. As the posterior end of groove A comes back posteriorly and is continuous in orientation, the lunate sulcus would have had to be in a more posterior orientation than in *Pan* brains. There are no landmarks at the posterior end of groove A which can be interpreted as a pongid-like lunate sulcus, and it is well established that only a minority of modern human brains show a distinct lunate sulcus^{10,16}. If and when it appears, it is in a very posterior position.

It can also be seen in Fig. 1 that no anterior-convex sulcus can be found at the anterior end of groove A which could be interpreted as a lunate sulcus. Furthermore, Fig. 1 shows a depression or groove (B) at the posterior end of groove A which is caused by the inferior lip of the parietal bone along the lambdoid suture, remnants of which are visible on the endocast. Thus, a posterior location for the lunate is possibly masked by the lambdoid sutural region, which is quite posterior in its location. No trace of a lateral calcarine from OP (occipital pole) to the sutural remnant can be found. This is not unusual, and these same patterns (or lack of them) are found in the same 140 pongid endocasts in my collection, which includes *Gorilla*, *Pongo* and *Hylobates*. C suggests the presence of a small triradiate depression on the left side, well medial to groove A. On *Pan* brain casts, and most published illustrations, a triradiate dimple is commonly found medial to the interparietal sulcus. All of this supports the identification of groove A as representing the interparietal sulcus and not the lateral calcarine fissure.

The transverse sinus cannot be discerned between the occipital and cerebellar lobes, nor is the fragment complete enough to detect any marginal or accessory sinus which would occur lateral to the foramen magnum, a condition which does appear on two other Hadar endocast specimens: the adult AL 333-45, and the infant AL 333-105 (see below).

Most simply, the morphology of the Hadar 162-28 endocast suggests that expansion of parietal association cortex and the diminution of lateral extent of primary visual striate cortex had occurred some few million years ago. This would be unequivocal evidence for early brain reorganization if hominids and *Pan*-like pongids split phylogenetically >5 Myr ago. An alternative hypothesis would be that hominids and pongids shared a basically hominid cerebral pattern, and modern *Pan* evolved its own pattern in the recent evolutionary past. This seems unlikely, given the pattern on other pongid brains, but we have no firm

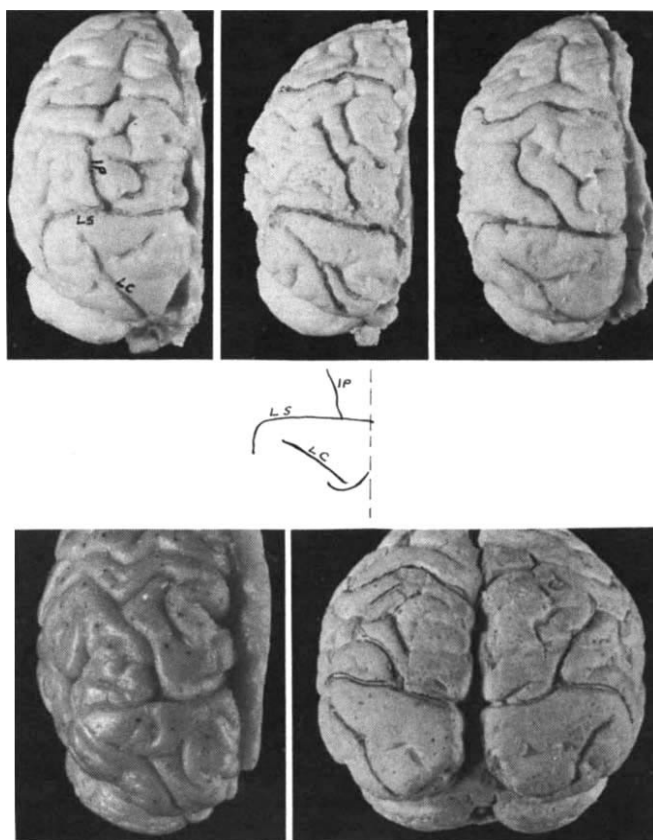


Fig. 2 A composite photograph made up of the dorsal and posterior views of five *Pan troglodytes* chimpanzee brain casts for comparison with the AL 162-28 fossil endocast (Fig. 1). These follow the numbers on Table 1, from top left to bottom right. Note the invariant morphological pattern of the relationships between the interparietal (IP), lateral calcarine (LC) and lunate sulcus (LS). The anterior end of LC is always bounded by the LS, and is always well lateral to IP. The IP always abuts posteriorly on the LS in *Pan* brains (photographs by author). Photographs of line drawings of *Gorilla*, *Pongo* and *Hylobates*, show the same pattern^{9,12}.

secure evidence regarding the evolution of the pongid brain. The 'Proconsul' endocast fragment strongly suggests an anteriorly located lunate sulcus¹⁷, so the third possibility, that this specimen possessed an unusual, unnamed posterior cerebral organization not present in either pongids (fossil or extant) or hominids seems unlikely.

If this analysis is correct, the significance of these preliminary findings is very important. The consensus opinion¹⁸⁻²⁰ appears

to be that the brain was the last element to change in human mosaic evolution. This should be corrected to mean 'brain size only', and not 'the brain'. It means that by 3–4 Myr ago, natural selection which would lead to enlargement of the parietal cerebral cortex was beginning to occur. It further suggests that the development of bipedal locomotion was integrated with neuropsychological restructuring.

These findings also bear on questions of punctuated equilibria and gradualism. It seems that plotting brain sizes against time cannot adequately take into account either cerebral reorganization, changes in relative brain size (we do not know if the Hadar specimens were more 'encephalized' than *Pan* species), or hierarchical timing of neurogenic events. Changes in cranial capacity, or brain volume are therefore inadequate and very distal phenotypic characteristics in understanding real evolutionary dynamics^{21,22}. Subsequent brain enlargement, however, is well documented after the australopithecines^{20,22,23}.

Finally, some additional observations relative to the taxonomic position of *Australopithecus afarensis*, *Australopithecus africanus* and *Australopithecus robustus* should be considered. As mentioned above, no evidence can be found for

a marginal sinus on the AL 162-28 specimen, although it does occur very clearly on the adult AL 333-45 and infant AL 333-105 *afarensis* specimens. This pattern was found and described by Tobias²⁴ on the OH 5 *Australopithecus boisei* endocast. It also appears on SK 1585²⁵ and SK 849 from Swartkrans, South Africa, and ER 407, 732, probably females of *A. boisei*, from Lake Turkana, Kenya²². None of the endocasts considered as *A. africanus*, either from South Africa, Kenya or Ethiopia (for example, Omo 338 s²³), shows such a pattern of venous drainage. It cannot be found in any of over 120 *Pan* or *Gorilla* endocasts in my collection. The hypothesis put forward by Johanson *et al.*⁴ regarding the closer affinities of *A. afarensis* to *A. africanus*, and the subsequent evolutionary split of robust lineages from *A. africanus* is not supported by the above observations, but rather suggests that we have yet to find early robust australopithecine forms within the 1.8–3.0 Myr time range, particularly given the new dating by Brown *et al.*⁵.

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1. Dart, R. *Nature* **115**, 195–199 (1925).
2. Falk, D. *Am. J. phys. Anthropol.* **53**, 525–539 (1980).
3. Holloway, R. L. *Am. J. phys. Anthropol.* **56**, 43–58 (1981).
4. Johanson, D. C., Taieb, M. & Coppens, Y. *Am. J. phys. Anthropol.* **57**, 373–402 (1982).
5. Brown, F. H. *Nature* **300**, 631–632 (1982).
6. Kimbel, W. H., Johanson, D. C. & Coppens, Y. *Am. J. phys. Anthropol.* **57**, 453–500 (1982).
7. Cronin, J. E., Boaz, N. T., Stringer, C. B. & Rak, Y. *Nature* **292**, 113–122 (1981).
8. Eldredge, N. & Tattersall, I. *The Myths of Human Evolution* (Columbia University Press, New York, 1982).
9. Holloway, R. L. in *Sensory Systems in Primates* (ed. Noback, C. R.) 181–200 (Plenum, New York, 1978).
10. Connolly, C. J. *External Morphology of the Primate Brain* (Thomas, Illinois, 1950).
11. Clark, W. E. LeGros, J. *Anat.* **81**, 300–333 (1947).
12. Smith, G. E. *Mott Memorial Volume* 57–65 (Adlard, London, 1929).
13. Retzius, G. *Das Affenhirn* (Fischer, Jena, 1906).
14. Shantha, T. R., Manocha, S. L. in *The Chimpanzee* Vol. 1 (ed. Bourne, G. E.) 188–293 (University Park Press, Baltimore, 1969).
15. Holloway, R. L. *Phil. Trans. R. Soc. Lond. B* **292**, 155–166 (1981).
16. Levin, G. *Am. J. phys. Anthropol.* **22**, 345–356 (1937).
17. Radinsky, L. *Am. J. phys. Anthropol.* **41**, 15–28 (1974).
18. White, T. D. *Science* **208**, 175–176 (1980).
19. McHenry, H. M. *Science* **190**, 425–431 (1975).
20. Falk, D. *Yb. phys. Anthropol.* **23**, 93–108 (1980).
21. Holloway, R. L. in *Development and Evolution of Brain Size* (eds Hahn, M. E., Jensen, C. & Dudek, B. C.) 59–88 (Academic, New York, 1979).
22. Holloway, R. L. *Can. J. Anthropol.* (in press, 1983).
23. Holloway, R. L. *Am. J. phys. Anthropol.* **54**, 109–118 (1981).
24. Tobias, P. V. *Olduvai Gorge* Vol. 2 (Cambridge University Press, 1967).
25. Holloway, R. L. *Am. J. phys. Anthropol.* **37**, 173–186 (1972).

Giant squids may die when exposed to warm water currents

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Strandings of the giant squid, *Architeuthis monachus* (Steenstrup), have always stirred attention because of the rarity and enormous size of these cephalopods. These animals have never been observed in their natural habitat and little is known about their physiology and ecology. Stranding of giant squids in Newfoundland waters has been correlated with the inflow of warm water, suggesting that increased temperature may be causing their death¹. Squids have also been carried to the Norwegian coast with the warm North Atlantic current² and on 23 August 1982 a live specimen was caught off Radøy near Bergen, Norway (Fig. 1). This catch gave an unprecedented opportunity to study the effects of temperature on the oxygen binding properties of blood from the giant squid. The present finding of an excess of a fourfold decrease in O₂ affinity when temperature is increased from 6.4 to 15 °C strongly suggests that giant squids may suffocate from arterial desaturation when increased ambient temperatures are experienced.

The animal had been dead for 2 days before a blood sample was taken from the efferent branchial artery. The blood sample was centrifuged to remove cells and debris, and filtered through a bacterial filter (0.45 µm), then the blood was stored in iced water ready for experimental procedures.

The Cl⁻ ion concentration of the blood was 452 mM, [Ca²⁺] 8.5 mM, [Mg²⁺] 41 mM, [Na⁺] 385 mM, [K⁺] 25 mM, osmolality 890 mOsm. These values differ less than 10% from similar data obtained from 13 live specimens of the common squid, *Todarodes sagittatus* (O.B., unpublished). A dilution of haemocyanin thus seems unlikely. The protein concentration of the blood was 22 mg ml⁻¹, giving an O₂ carrying capacity of

about 0.3 mM O₂ assuming a molecular weight of 4,500 and 80 O₂ binding sites per mole protein³.

The relationship between CO₂ tension and pH in oxygenated and deoxygenated blood was determined by measuring the pH of blood equilibrated with the appropriate gas mixtures in a tonometer kept at constant temperature. Above a P_{CO2} of 1.5 mm Hg there was no difference in pH between oxygenated and deoxygenated blood. Below this CO₂ tension the pH of oxygenated blood was slightly higher (0.04 units) than the pH of deoxygenated blood. The influence of the Haldane effect shifting pH during oxygenation would thus be minimal as could be expected from the very low haemocyanin concentration measured.

O₂ dissociation curves at a constant P_{CO2} (pH ~ constant) were measured spectrophotometrically at 366 nm and at 6.4, 10 and 15 °C (ref. 4). The results demonstrate a very pronounced temperature sensitivity of the O₂ affinity of the blood of *A. monachus*. The heat of oxygenation, ΔH, which includes the heat of solution of oxygen (~ -3.1 kcal per mole at 15 °C) was about -19 kcal per mole. This value is very similar to those obtained for blood from the Antarctic fish, *Trematomus bernacchi*⁵, which lives at very constant ambient temperatures, and about twice as high as the values found for herring, trout, bowfin, plaice and flounder⁶.

Assuming an arterial O₂ tension (P_{O2}) of 90 mm Hg, which may be typical of cephalopods⁷, we may assess the effect on O₂ affinity of an increase in ambient temperature from 6.4 °C (close to assumed habitat temperature) to 15 °C (ambient temperature at time of capture).

At 6.4 °C the postbranchial blood would be more than 90% saturated at a P_{O2} of 90 mm Hg (P_{CO2} = 0.7 mm Hg; P₅₀, P_{O2} at half saturation, = 27 mm Hg). Assuming similar changes in P₅₀ with pH (Bohr factor = Δ log P₅₀/Δ pH) as demonstrated for blood at 10 and 15 °C in the P_{CO2} range 0.074 to 7.4 mm Hg, we can estimate that the blood would have been nearly fully saturated at a P_{O2} of 90 mm Hg over the entire P_{CO2} range. An increase in P₅₀ of more than fourfold occurred when the temperature was increased from 6.4 to 15 °C. The haemocyanin

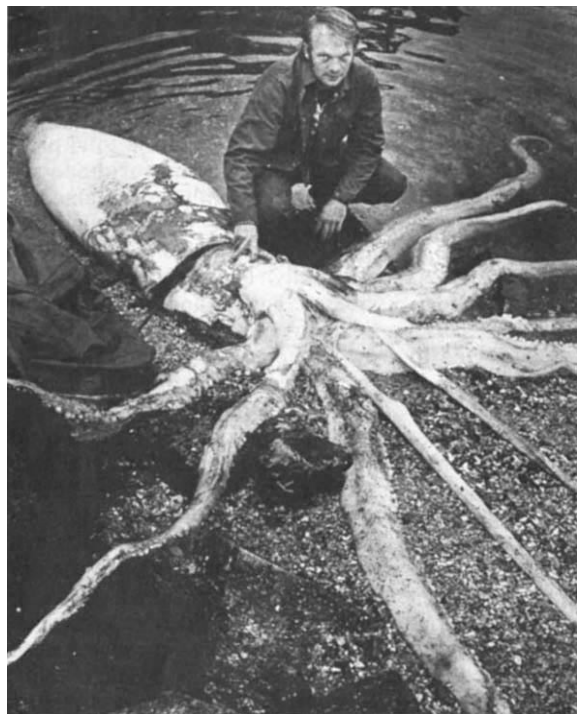


Fig. 1 The fisherman and his catch. The giant squid was hooked in a 5 m deep small bay. The squid was dying at the time of capture. Total length was about 10 m, the tentacles were about 7.3 m, and the weight about 220 kg (photograph: J. M. Lillebøe, B.T., Norway).

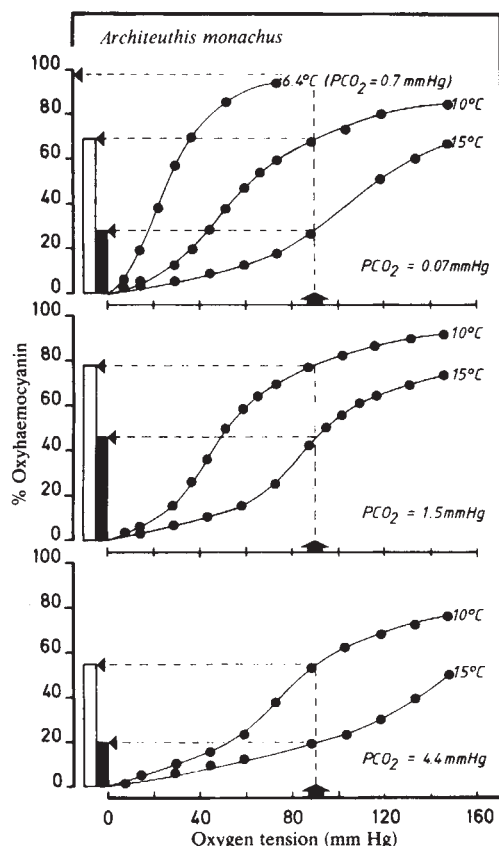


Fig. 2 Blood oxygen dissociation curves obtained at constant P_{CO_2} s of 0.07 mm Hg ($pH = 7.93$), 1.5 mm Hg ($pH = 7.60$), and 4.4 mm Hg ($pH = 7.30$) at 10 and 15°C. P_{CO_2} was 0.7 mm Hg ($pH = 7.73$) at 6.4°C. The large arrows indicate the typical cephalopod arterial P_{O_2} of 90 mm Hg⁷. The bars show estimated arterial O_2 saturations at 10°C (open bar), and 15°C (filled bar) assessed from the O_2 equilibrium curves measured for *A. monachus* blood.

would thus be less than half loaded with O_2 at 15°C and a P_{CO_2} of 1.5 mm Hg, whereas at 10°C the pigment would still be about 80% saturated (Fig. 2).

Increased activity may raise blood P_{CO_2} . At a P_{CO_2} of 4.4 mm Hg the O_2 saturation would decrease to about 55% at 10°C, and it may be that the O_2 requirement could not be satisfied at this temperature taking into account the very low O_2 carrying capacity of the haemocyanin (0.3 mM O_2). At 15°C less than one-fifth of the haemocyanin would be loaded with O_2 . Even a relatively small increase in ambient temperature may thus have a very serious effect on tissue O_2 provision in the giant squid due to the pronounced temperature sensitivity of its blood.

Hyperventilation decreasing the general level of blood P_{CO_2} will normally favour O_2 loading in the gills of cephalopods because of the high pH sensitivity of their blood (Bohr shift)⁷⁻⁹. *A. monachus* blood as shown here, however, has a reversed Bohr shift below a P_{CO_2} of 0.7 mm Hg, a feature not previously recorded for cephalopod blood (Fig. 2). Hyperventilation and a respiratory alkalosis would thus further impair O_2 loading.

The high temperature sensitivity of the O_2 affinity in combination with the very low O_2 carrying capacity of the blood could thus be a cause of death for giant squids experiencing higher than normal water temperatures along the shores of Newfoundland, Great Britain and Scandinavia in the warm Atlantic current, which can in some years carry large volumes of warm water northwards.

The O_2 carrying capacity demonstrated in the present study for the giant squid is very low compared with the active oceanic cephalopods (1.6 to 1.9 mM O_2 , ref. 10). This feature suggests that the giant squid is a relatively poor swimmer and a passive and sluggish predator¹¹.

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1. Aldrich, F. A. *Sarsia* **34**, 393-398 (1968).
2. Kjennerud, J. *Naturvit. rekke, Univ. Bergen* **9**, 2-14 (1958).
3. Gliens, C., Preux, G. & Lontie, R. *Eur. J. Biochem.* **60**, 271-280 (1975).
4. Lykkeboe, G. & Johansen, K. *J. comp. Physiol.* **104**, 1-11 (1975).
5. Grigg, G. C. *Comp. Biochem. Physiol.* **23**, 139-148 (1967).
6. Everaarts, J. M. *Netherl. J. Sea Res.* **12**, 1-57 (1978).
7. Johansen, K., Brix, O. & Lykkeboe, G. *J. exp. Biol.* **99**, 331-338 (1982).
8. Lykkeboe, G., Brix, O. & Johansen, K. *Nature* **287**, 330-331 (1980).
9. Brix, O., Lykkeboe, G. & Johansen, K. *Resp. Physiol.* **44**, 177-186 (1981).
10. Lykkeboe, G. & Johansen, K. *Pacif. Sci.* **33**(3), 305-313 (1983).
11. Roper, C. F. E. & Boss, K. J. *Scient. Am.* **246**, 82-89 (1982).

Growth of 'black smoker' bacteria at temperatures of at least 250 °C

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Complex communities of thermophilic bacteria have been cultured from the 350 °C waters emanating from sulphide chimneys, or 'black smokers', at 21 °N along the East Pacific Rise¹. Several of the bacterial communities were shown to grow rapidly at 100 °C and atmospheric pressure, producing methane, hydrogen gas and carbon monoxide. These gases are found in superheated vent water, having previously been attributed to abiogenic reactions. Before concluding that these 'black smoker' bacteria actually contribute to the chemistry of the superheated hydrothermal fluids, it was necessary to test their ability to grow and produce gases at *in situ* vent tem-

peratures and pressures. Here we report that a bacterial community originally cultured from 306 °C water is capable of chemolithotrophic growth in a titanium growth chamber under *in situ* vent pressure of 265 atm and at temperatures of at least 250 °C. (At 265 atm, seawater remains liquid at temperatures of at least 460 °C².) Transmission electron microscopy of thin sections of bacteria cultured at 250 °C has revealed the presence of at least two morphologically distinct organisms.

The upper temperature limit of environments known to support life has rarely been reported as exceeding 100 °C, although there have been reports of bacteria existing in hot waters at temperatures a few degrees above 100 °C³⁻⁷, or cultured at 105 °C⁷. Only prokaryotic organisms are found in environments hotter than 75 °C, and at temperatures above 85 °C microbial diversity decreases sharply³. A stable microbial habitat at temperatures exceeding 100 °C requires liquid water, and therefore must be under either hydrostatic or osmotic pressure. Such environments exist on Earth, but none are so dramatic as the deep-sea hydrothermal systems recently discovered along submarine tectonic rifts and ridges^{8,9}. One of the vent types found along the East Pacific Rise, and at the Gorda Ridge and the Guaymas Basin, is characterized by conical sulphide chimneys ('smokers') out of which spew superheated waters at temperatures exceeding 350 °C⁸⁻¹⁰. These waters contain supersaturated levels of reduced gases and metals^{8,11-13}, potential energy sources for bacteria which serve as the primary producers in the vent food chains^{8,14}. They also contain complex thermophilic microbial communities capable of rapid growth and gas production at 100 °C and atmospheric pressure¹. We were prompted to incubate one of the intact communities at even higher temperatures and pressure to determine whether the bacteria were capable of growth in *in situ* vent conditions (350 °C and 265 atm).

The apparatus used to monitor the microbial community at high temperature and pressure, without cooling or decompressing the primary culture, was a modification of an earlier design described by Yayanos¹⁵ (see Fig. 1 legend). The bacterial content of subsamples was estimated by epifluorescent microscopy, after staining with the DNA-specific dye 4',6'-diamidino-2-phenylindole 2 HCl (DAPI). Protein concentrations in duplicate subsamples were determined by both the Folin-Lowry and Coomassie blue methods.

Growth curves of these mixed cultures at 150, 200, 250 and 300 °C are shown in Fig. 1. Bacterial doubling times were 8 h at 150 °C, 1.5 h at 200 °C and 40 min at 250 °C. At 250 °C the protein concentration doubled at the same rate as the number of bacteria. Incubation at 265 atm clearly expanded the temperature range for growth, and required at least 250 °C for optimum growth rates equivalent to those at 100 °C and atmospheric pressure. At 300 °C, an apparent short growth spurt was followed by a decline in the number of single bacteria (Fig. 1d). During this decline clumps of a few to several hundred cells began to appear, and membranous spheres ranging in size from less than 1 to 10 µm occurred singly or in clumps of 20 or more (see Fig. 2d). DAPI-stainable particles were associated with these spheres. Concomitant with the appearance of clumps and membranous spheres was an increase in the level of particulate protein, which continued throughout the course of the experiment. Concentrations of soluble DNA, ranging from less than 1 µg to 5 µg DNA per ml, were also detected in the medium during this time. One possible interpretation of these data is that bacteria continue to reproduce at 300 °C, as shown by the microscopic observations and increase in protein concentrations, but that lysis occurred during the decompression required to collect the sample releasing DNA. Some lysis may also have occurred at 265 atm as a result of marked decrease in pH which is known to occur in seawater at 300 °C under pressure¹⁶. An uninoculated medium control incubated at 200 °C for 16 h contained no DAPI-stainable particles. When mercury was introduced into an inoculated control incubated in similar conditions, the number of DAPI-stainable cells decreased at a rate of approximately 1 log unit per 30 min (Fig. 1b).

The amino acid composition of total protein extracted from cells in late log growth phase at 250 °C is shown in Table 1, primarily to substantiate that the colorimetric methods were measuring complete protein. Almost all of the amino acids were detected, although there were five unknown amino acid and/or peptide peaks that represented over 25% of the total protein. The unusually high levels of glycine, glutamic acid and serine in this protein preparation cannot presently be explained.

Electron micrographs of thin sections of the bacteria cultured at 250 °C illustrate the morphological diversity of this unique thermophilic microbial community. The bacteria shown in Fig. 2 were present in high numbers in late log and stationary growth stages at all temperatures examined. Most known bacteria that might be expected to grow in the extreme conditions of submarine hydrothermal vents are archaeobacteria, and include the methanogens, *Thermoplasma* and *Sulfolobus*^{3,17}. Although we did not observe organisms that lacked a cell wall, we noted other structures characteristic of some archaeobacteria such as cavitations, internal membranes with subunits (Fig. 2a,b), and thick cell walls (Fig. 2c)^{3,18-21}. The organisms in Fig. 2c also show a morphological resemblance to the thermophilic anaerobic microbe recently isolated from a shallow submarine solfatar environment and described by Stetter⁷. It is quite probable, however, that bacteria obtained from superheated vent waters are adapted for growth in the unusually extreme conditions found in submarine hydrothermal systems and thus possess unique structures, macromolecules and methods of replication.

The presence of methanogens in the inoculum we used for these high temperature/pressure growth studies was shown by initial experiments conducted at 100 °C and atmospheric pressure¹. Some of the bacteria cultured at 250 °C and 265 atm autofluoresced at 420 nm, which is presumptive evidence of a coenzyme found only in methanogenic bacteria²². A gas sample from a 3 h culture at 300 °C contained 9.6% CH₄, 2.2% H₂ and 0.1% CO, none of which were detected in the uninoculated medium control at 200 °C.

These findings open up the possibility that bacteria may exist and grow within the Earth's crust at temperatures exceeding

Table 1 Amino acid analysis of total protein from a 6 h culture at 250 °C and 265 atm pressure

Amino acid	Amino acid residues ($\times 10^{-3}$)
Aspartic acid	78.0
Threonine	36.8
Serine	105.7
Glutamic acid	116.0
Proline	46.5
Glycine	332.5
Alanine	69.0
Half-cystine	5.5
Valine	25.9
Methionine	9.8
Isoleucine	24.0
Leucine	45.9
Tyrosine	4.7
Phenylalanine	20.2
Histidine	17.1
Lysine	23.6
Arginine	39.0

Bacterial cells from a 0.25 ml sample of the 250 °C culture were concentrated by centrifugation using a Beckman Microfuge B for 1 min. The protein was precipitated using 5% trichloroacetic acid as described for Fig. 2. The protein was dialysed in distilled water, lyophilized, and hydrolysed with 6.1 M HCl for 22 h at 110 °C in an evacuated sealed tube. The sample was analysed using a modified Beckman 120 B amino acid analyser. The sample contained approximately 200 µg protein as determined by colorimetric methods (see Fig. 1 legend). In addition to the amino acids listed, there were five unknown amino acid peaks. These unknown amino acids and/or peptides represented over 25% of the total protein analysed. Tryptophan, cysteine and glutamine could not be determined quantitatively by this method.

Fig. 1 Growth curves of a 'black smoker' bacterial community and associated increases in total protein at temperatures of 150 °C (a), 200 °C (b), 250 °C (c) and 300 °C (d), under hydrostatic pressure of 265 atm. In b, * shows a mercury (Hg)-killed control experiment. An uninoculated medium control was also incubated and sampled at 200 °C for a period of 16 h. During this time period no DAPI-stainable particles were observed in any of the collected samples. In the uninoculated control, as well as in all of the growth experiments, evidence of H₂S and SO₂ production was noted by odour. Arrows in d indicate bacterial counts at the times when specific temperatures were reached. Error bars on the initial and following point at 300 °C represent variations in counts from three different dilutions of each sample. Fifty fields were counted per sample dilution. The subsequent decrease in cell number was accompanied by an increase in the number of single and multiple membrane structures varying in size from less than 1 to 10 µm. These spheres contained small (0.2 µm) DAPI-stainable particles.

Methods: The culture was obtained originally from a 306 °C water sample collected at a depth of 2,650 m during the October–November 1979 RISE expedition to 21°N along the East Pacific Rise. Further details on the characteristics of the bacterial community along with culture and maintenance procedures have been described elsewhere¹.

The growth medium used was minimal salts¹ supplemented with 0.2% Na₂S₂O₃·5H₂O; 0.02% MnSO₄·H₂O; 0.001% FeSO₄·7H₂O; 0.03% (NH₄)₂SO₄; 0.001% Ca(NO₃)₂; 0.05% Na₂HPO₄; 0.5% NaHCO₃; trace element mix¹; 0.001% Bacto-yeast extract; 0.3% HEPES buffer; distilled water, and adjusted to pH 6.5. The bacterial community was cultured at 100 °C in a water or sand bath for 48 h and diluted in fresh medium preheated to ~90 °C before each high temperature/pressure experiment. Bacterial cultures were loaded into a custom-made titanium syringe with a maximum volume of 120 ml. (A 50-ml glass syringe was used in the 150 °C growth experiment.) The syringe barrel is 20 cm long and 2.5 cm wide and consists of a titanium plunger fitted with a Teflon O-ring on the front end, and a Viton O-ring on both ends. The syringe was placed into a 750 ml pressure vessel containing high-temperature test silicon hydraulic fluid preheated to approximately 90–95 °C. The pressure vessel was entirely encased in a heating jacket. The syringe was fitted onto the valve cap of the pressure vessel. The syringe/pressure vessel/cap combination was then connected to pressure pumps and to the sample collecting vessel using high pressure-tested stainless steel tubing. Separate pressure pumps were used to apply equivalent pressures to the main culture pressure vessel and to the subsample pressure vessel. The sample fluid collected was at ambient temperature as a result of a temperature gradient in the stainless steel tubing. Before collecting a sample for analysis, 1.5–2 ml were transferred and discarded to ensure that residues of the previous samples were washed out of the stainless steel tubing. Approximately 2–4 ml of samples were usually collected for analysis. The cultures were collected in acid-cleaned test-tubes and immediately fixed with 2% (w/v) glutaraldehyde and maintained at 2–4 °C until counted. The pressure vessel was heated by a 140 V Variac, and the temperature regulated by F & M Model 220 power proportioning temperature controller. The temperature in the pressure vessel was measured by a thermistor-regulated controller as well as by a metal thermometer, both of which are inserted into the heating jacket. Generally, it took 35–39 min for a 50 °C increase within the range of 100–300 °C. The reaction vessel was pressurized to 265 atm before increasing the temperature, so as to avoid boiling the sample. The pressure was constantly monitored and adjusted during the temperature ascent period. When the desired temperature was reached, a time zero sample was taken. Total bacterial counts were measured by epifluorescent microscopy using the procedures of Porter and Feig²³. Cells were stained with the DNA specific fluorescent dye 4',6-diamidino-2-phenylindole 2HCl (DAPI). This dye binds specifically to adenine–thymine base pairs and has the advantage over acridine orange in that it does not interfere with other organic and inorganic compounds. Fixed cells were stained with DAPI to a final concentration of 0.02 µg ml⁻¹ for 5 min. The stained cells were concentrated on 0.2 µm Nucleopore filters prestained with Irgalan black dye. Protein was extracted and measured using the procedures described by Gallant and Suskind²⁴. Bacteria from two 0.5 ml samples were concentrated by centrifugation. Protein was precipitated in cold 5% trichloroacetic acid, solubilized in 1M NaOH, and measured by both the Folin–Lowry and Coomassie blue methods. For the Hg-killed control (b), 3 ml of Hg was pumped into the culture when the temperature reached 200 °C. Within 5 h there were fewer than 10² DAPI-stainable bacteria per ml in the sample. At the present time we cannot explain the mechanism of Hg disinfection at high temperature and high pressure, although it is quite likely that some Hg²⁺ ions are formed, as the medium in these conditions would be acidic¹⁶. We used this method for controls because a metallic Hg displacement vessel was used for collecting samples from the high temperature/high pressure growth chamber and so it was easy to transfer Hg into the culture.

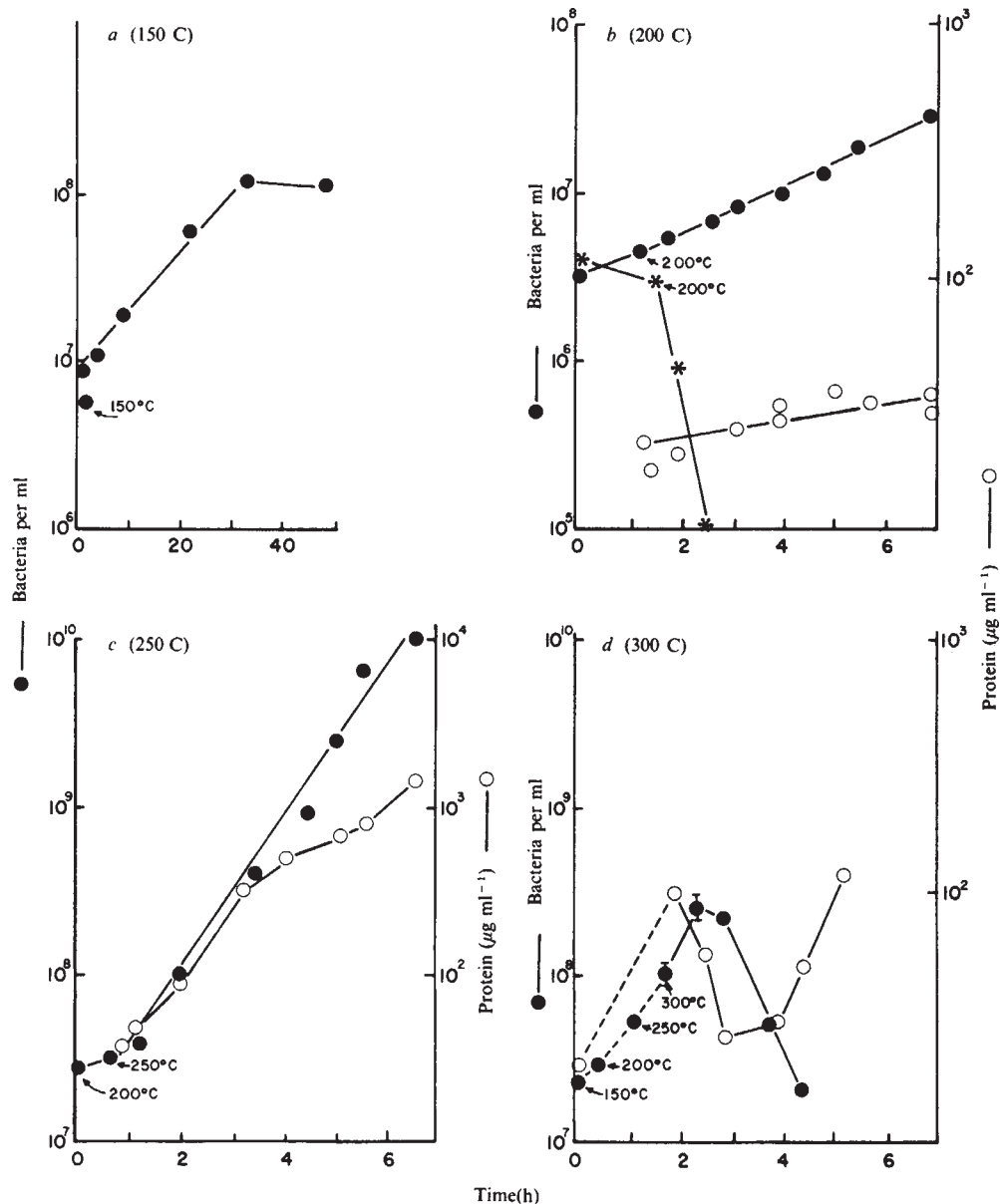
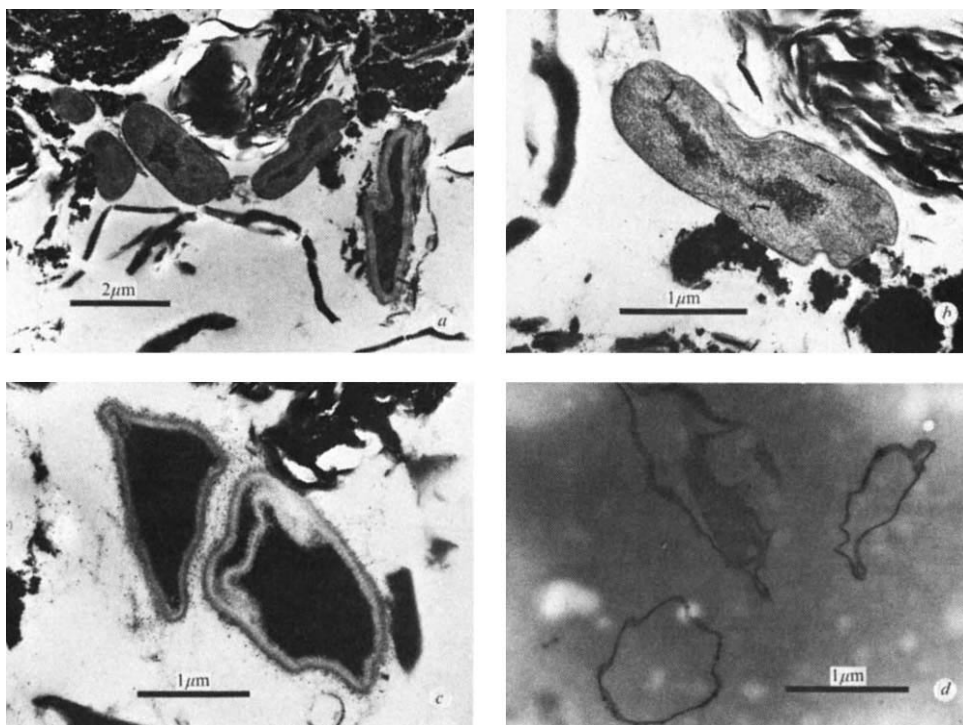


Fig. 2 Transmission electron micrographs of ultrathin sections of microorganisms commonly observed in the 150–300 °C samples. Samples were taken from a 7 h culture at 250 °C and 265 atm (a–c). The two most abundant rod-shaped bacteria are shown in a. Note the internal membranes (mesosomes) in the more abundant organisms, and the thick cell wall and lack of any definable internal structures in the bacterium on the right. These same two organisms are shown at higher magnification in b and c. Note the pleomorphism in both organisms. The internal wall-membrane complex seen in c is probably a result of sectioning through two planes of one organism. Vertical subunits are seen clearly in this internal cell wall-membrane complex. Thin sections of the spheres formed after 3 h at 300 °C are shown in d. The particulate protein measured in the 300 °C culture was derived from these spheres as observed microscopically.

Methods: The cultures were subsampled from the pressure vessel and fixed immediately in glutaraldehyde (2.5% final concentration). The fixed cells were concentrated by centrifugation for 1 min. The pellet was resuspended in 0.2 M cacodylate buffer at pH 7.8. Cells were dehydrated in acetone, stained with 1% OsO₄ and 1% uranyl acetate, and sectioned.



250 °C. As the thermophilic bacterial community examined in this study has a wide range of physiological activities, including formation and utilization of oxidized and reduced metals, sulphur and trace gases, hypotheses related to the origin of these elements and compounds in submarine hydrothermal systems may need to be reassessed.

Finally, these results substantiate the hypothesis that microbial growth is limited not by temperature but by the existence of liquid water, assuming that all other conditions necessary for life are provided. This greatly increases the number of environments and conditions both on Earth and elsewhere in the Universe where life can exist.

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- Baross, J. A., Lilley, M. D. & Gordon, L. I. *Nature* **298**, 366–368 (1982).
- Chen, C.-T. A. *Science* **211**, 298 (1981).
- Brock, T. D. *Thermophilic Microorganisms and Life at High Temperatures* (Springer, New York, 1978).
- Tansey, M. R. & Brock, T. D. in *Microbial Life in Extreme Environments* (ed. Kushner, D. J.) 159–216 (Academic, London, 1978).
- ZoBell, C. E. *Producers Mon. Penn. Oil Prod. Ass.* **22**, 12–19 (1958).
- Vidal, V. M. V., Vidal, F. V. & Isaacs, J. D. *J. geophys. Res.* **83**, 1757–1774 (1978).
- Stetter, K. O. *Nature* **300**, 258–260 (1982).
- Corliss, J. B. *et al. Science* **203**, 1073–1083 (1979).
- Spies, F. N. *et al. Science* **207**, 1421–1423 (1980).
- Curry, J. R. *et al. GeoTimes* **24**, 18–20 (1979).
- Lilley, M. D., de Angelis, M. A., & Gordon, L. I. *Nature* **300**, 48–50 (1982).
- Edmond, J. M., Von Damm, K. L., McDuff, R. E. & Measures, C. I. *Nature* **297**, 187–191 (1982).
- Welhan, J. A. & Craig, H. *Geophys. Res. Lett.* **6**, 829–831 (1979).
- Karl, D. M., Wirsén, C. O. & Jannasch, H. W. *Science* **207**, 1345–1347 (1980).
- Yayanos, A. A. *Rev. scient. Instrum.* **40**, 961–963 (1969).
- Bischoff, J. L. & Seyfried, W. E. *Am. J. Sci.* **278**, 838–860 (1978).
- Woese, C. R. *Zentbl. Bakt. Hyg., I. Abt. Orig. C3*, 1–17 (1982).
- Stetter, K. O. *et al. Zentbl. Bakt. Hyg., I. Abt. Orig. C2*, 166–178 (1981).
- Huber, H., Thomm, M., König, H., Thies, G. & Stetter, K. O. *Arch. Mikrobiol.* **132**, 47–50 (1982).
- Zeikus, J. G. & Wolfe, R. S. *J. Bact.* **113**, 461–476 (1973).
- Kandler, O. L. *Zentbl. Bakt. Hyg., I. Abt. Orig. C3*, 149–160 (1982).
- Doddema, H. J. & Vogels, G. D. *Appl. envir. Microbiol.* **36**, 752–754 (1978).
- Porter, K. G. & Feig, Y. S. *Limnol. Oceanogr.* **25**, 943–948 (1980).
- Gallant, J. & Suskind, S. R. *J. Bact.* **82**, 187–194 (1961).

Variable expression of Ia antigens on the vascular endothelium of mouse skin allografts

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Ia antigens are membrane-bound glycoproteins that play a part in antigen recognition and subsequent cell-cell interactions in the immune response. In the mouse they are coded for by the *I* region of the major histocompatibility complex *H-2* and have been demonstrated on B lymphocytes, monocytes, activated T cells, macrophages and dendritic cells, including Langerhans cells^{1,2}. Ia-like antigens have also been detected on the vascular endothelium in man^{3–6} and on epidermal keratinocytes in rats^{7,8} but expression on the latter cells was induced by a graft-versus-host reaction or by contact hypersensitivity⁹. In the mouse, previous studies have suggested that Ia antigens in skin are restricted to epidermal Langerhans cells and it was thought that these were the targets for Ia-dependent rejection of skin allografts. The results presented here show that Ia antigens in mouse allografts are also present on the vascular endothelium but their expression is variable and dependent on the immunological status of the recipient. These findings suggest that vascular endothelial cells can act as targets in Ia-incompatible skin allograft rejection.

We investigated the presence of Ia antigens in mouse skin allografts and the localization of these antigens by *in vivo* injection of labelled anti-Ia alloantibodies. A monoclonal anti-Ia IgG2a alloantibody, raised in our laboratory in BALB/c (*H-2^d*) mice against BALB/K (*H-2^k*) donor cells, was used. The Ia^k determinant recognized by this antibody was shown to map in the *I* region by a positive binding reaction with 40–50%

of B10 . AQR (Ia^k) spleen lymphocytes and a negative reaction with B10 . T (6R) (Ia^d) cells. These strains differ only at the *I* and *S* region of the *H*-2 complex. The anti-Ia antibody was labelled with ¹²⁵I using the chloramine-T method¹⁰ and aliquots of approximately 5×10^4 c.p.m. were injected intravenously (i.v.) into otherwise untreated female B10 . D2 (*H*-2^d) recipients of B10 . BR (*H*-2^k) and BALB/c (*H*-2^d) tail skin allografts on day 6 after transplantation. The grafts were excised after different time intervals and the amount of radioactivity was determined by γ -counting of the entire graft.

Figure 1 shows that specific binding of the anti-Ia antibodies to the B10 . BR grafts occurred, with a maximum at 8–12 h after injection. In the BALB/c control grafts a background binding level was observed after each time interval. To localize the bound radioactive antibodies, B10 . BR skin grafts were excised 16 h after injection and prepared for autoradiography (see the legend to Fig. 2). Microscopic examination showed that the bound radioisotope was localized in the vessel walls of the donor skin (Fig. 2a). The vessels of the recipient, visible in a rim of tissue surrounding the graft, were negative in all cases. All other structures of donor origin, including the epidermis, were also negative. To identify the localization of the vessel wall associated Ia antigens more precisely, the monoclonal anti-Ia was labelled with biotin and the conjugate was administered as outlined above. After excision of the grafts cryosections were prepared and developed with avidin conjugated with fluorescein. The capillary endothelial cells were then

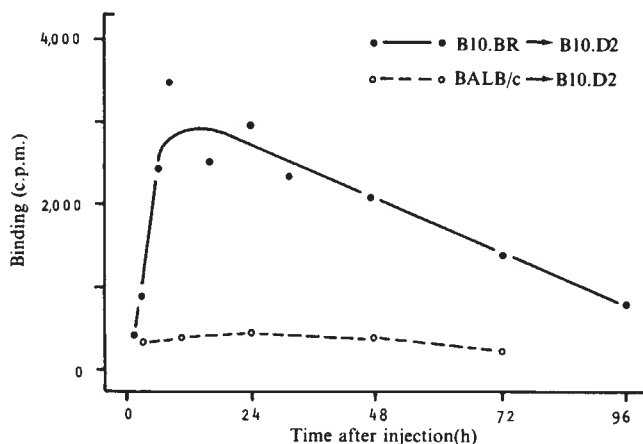
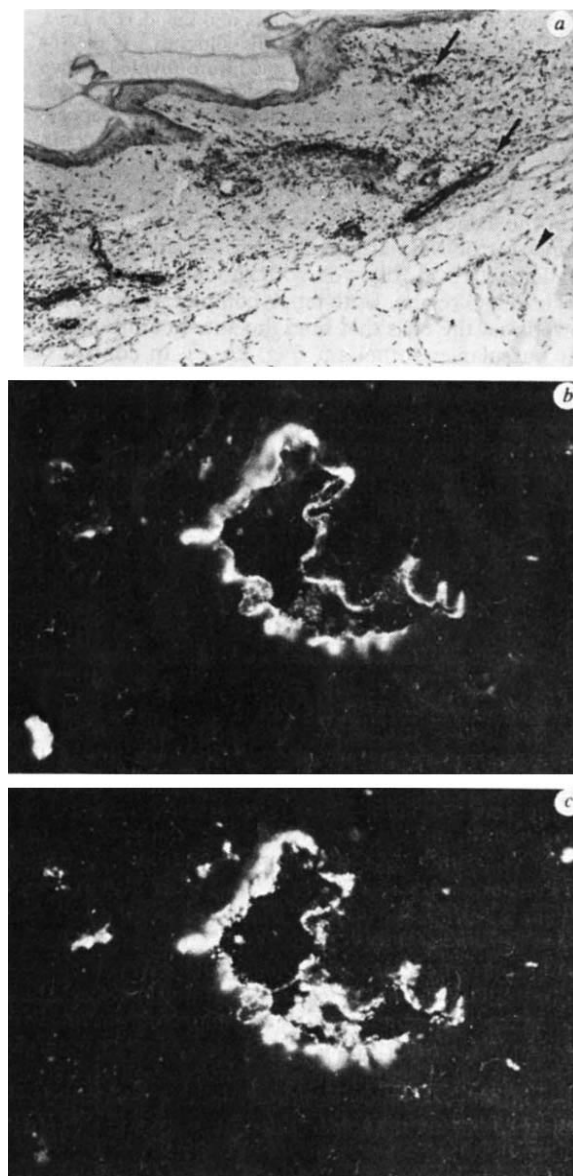


Fig. 1 Binding of ¹²⁵I-labelled anti-Ia^k monoclonal IgG2a to B10 . BR (●—●) and BALB/c (○—○) skin allografts *in vivo*. Female B10 . D2 recipients were injected intravenously on day 6 after transplantation with 0.1 ml phosphate-buffered saline containing 0.1% bovine albumin and 3.8×10^4 c.p.m. of radio-label. After different time intervals the recipients were killed, the grafts excised, and the amount of radioactivity determined. Each point represents the mean value of two measurements.

Fig. 2 a, Autoradiomicrograph showing the localization of ¹²⁵I-iodine-labelled monoclonal anti-Ia IgG2 in B10 . BR skin allografts after passive administration to BALB/c recipients *in vivo*. $\times 31$. Male recipient mice were injected intravenously at day 6 after transplantation with 0.1 ml phosphate-buffered saline (PBS) containing 0.1% bovine albumin and 17×10^4 c.p.m. of ¹²⁵I-labelled anti-Ia^k IgG2. Grafts were removed after 16 h and put in neutralized 4% formaldehyde solution. After fixation and imbedding, 5- μ m paraffin sections were cut and transferred to gelatinized slides. The sections were deparaffinated, rinsed in distilled water and dipped in photographic emulsion (Nuclear Research Emulsion, Ilford K5, 3 $\times$ diluted with water) in the dark. The slides were air-dried overnight in a humid atmosphere at room temperature and then left for 4 weeks in sealed boxes at 4 °C. After exposure the emulsion was developed with developing fluid (Kodak), and the sections were stained with haematoxylin and eosin. Photomicrographs were made with black and white film (Agfapan-25) using a green filter. The localization of the intravenously injected anti-Ia antibody is clearly visible as a positive staining of the vessel walls of the graft (arrows). The vessels of the recipient are negative (arrowhead). **b**, Micrograph showing the localization of biotin-labelled anti-Ia^k in a B10 . BR skin allograft after passive administration to a B10 . D2 recipient *in vivo*. The figure shows the presence of fluorescein isothiocyanate-labelled anti-Ia antibodies on the endothelium of the graft, demonstrating the presence of donor Ia^k antigens. $\times 78$. 1 mg of monoclonal IgG2a was conjugated with 120 μ g of the *N*-hydroxysuccinimide ester of biotin (Sigma) in 1 ml of 0.1 M NaHCO₃ at room temperature. 100 μ l of the conjugate were injected intravenously on day 6 after transplantation of both a B10 . BR and a BALB/c graft onto an untreated B10 . D2 recipient. After 2 h the grafts were excised and frozen in liquid nitrogen. Cryosections 4–5- μ m thick were air dried, fixed in acetone for 10 min and washed with PBS. The sections were then incubated in avidin-FITC (Sigma), diluted 500 $\times$ in PBS containing 0.1% bovine serum albumin (BSA), and washed again. Subsequently the tissue sections were incubated with rabbit antiserum against human factor VIII antigen (Red Cross, Amsterdam) diluted 100 $\times$, washed and developed with goat antiserum against rabbit IgG, conjugated with rhodamine (Cappel), diluted 50 $\times$. Finally the sections were rinsed, mounted in Aquamount (Gurr) and examined under a Leitz Dialux fluorescence microscope, equipped with Ploemopak epilluminator and filter combinations for fluorescein and rhodamine labels. **c**, Using the filters for rhodamine fluorescence, the binding of the anti-factor VIII antibodies is shown in the same section as in **b** demonstrating the presence of factor VIII antigen on the endothelium. $\times 78$.



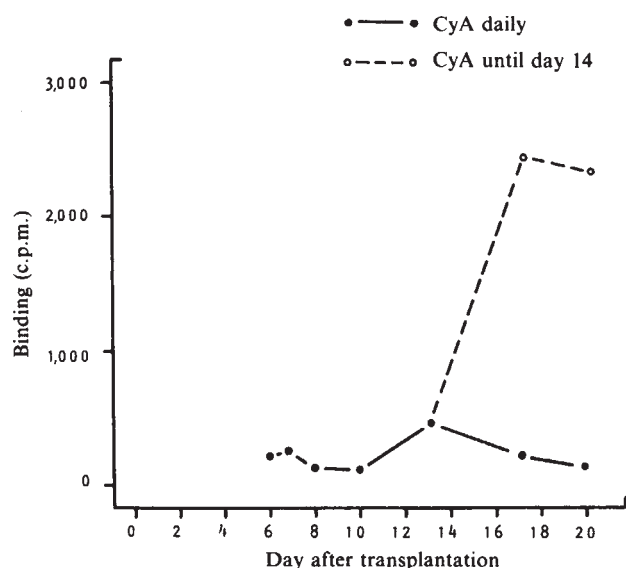


Fig. 3 Binding of ^{125}I -labelled anti Ia^k monoclonal IgG2a to B10.AQR skin allografts *in vivo*. Sixteen female B10.T(6R) recipients of B10.AQR grafts daily received 75 mg kg^{-1} cyclosporin A (Sandoz) orally, dissolved in olive oil at a concentration of 20 mg ml^{-1} . After different time intervals binding of radio-labelled anti- Ia^k was determined. For this, recipient mice were injected intravenously with 4.0×10^4 c.p.m. and killed 16 h later, to determine the amount of binding in the allograft. At day 14 after transplantation the remaining 8 mice were divided in two groups, one in which the immunosuppression was continued and one in which the administration of cyclosporin A was stopped. Most of the points represent the mean value of 2–3 measurements.

identified in the same sections by incubation with rabbit anti-serum against factor VIII antigen, which is a specific marker for endothelial cells^{11,12}, followed with rhodamine-conjugated goat anti-rabbit IgG serum. Both labels completely overlapped, which showed that the cells that bind the anti- Ia antibodies are cells of the vascular endothelium (Fig. 2b, c). In control skin allografts from BALB/c ($H-2^d$) donor mice, binding of biotinylated anti- Ia^k antibodies was undetectable. The binding of a control monoclonal antibody (WT3, raised against human T lymphocytes) that was injected into B10.D2 recipient mice carrying B10.BR allografts was also negative.

In these experiments we had observed a correlation between the binding of labelled anti- Ia antibodies and the degree of rejection. Grafts showing macroscopic signs of rejection bound considerably more antibody than healthy grafts. Perhaps expression of donor strain Ia antigens on the vascular endothelium of the allograft might be influenced by the immune response of the host. To investigate this, donor tail skin was transplanted onto immunosuppressed and nude recipients. *In vivo* binding studies were performed at day 7 after transplantation by i.v. injection of radiolabelled monoclonal anti- Ia antibody followed by excision and γ -counting of the skin grafts

16 h after injection. The results of these experiments are shown in Table 1. A high binding of anti- Ia antibodies was observed if donor skin from mice carrying Ia^k antigens (B10.BR or B10.AQR) was grafted onto untreated normal recipients. Background levels of binding were seen if the donor skin lacked the Ia^k antigens (B10.D2), even in the presence of an $H-2$ incompatibility between donor and recipient (B10.T(6R) $\rightarrow$ B10.D2). Similar results were obtained with the monoclonal anti-I-E/C^k antibody coded 14-4-4 which was prepared by Ozato *et al.*¹³ (data not shown). In recipients treated with immunosuppressive agents such as rabbit anti-mouse lymphocyte serum or cyclosporin A (CyA), there was also only background binding of radioactivity in skin grafts of Ia^k -positive donors, demonstrating the dependence of endothelial Ia antigen expression on the immune response of the host (Table 1). A binding inhibition assay showed that the anti-mouse lymphocyte serum did not contain anti- Ia xenoantibodies that could interfere with the *in vivo* binding studies (data not shown). If congenitally athymic BALB/c *nu/nu* mice, which lack a T-dependent immune system, were used as recipients of B10.BR grafts, binding of the radiolabelled anti- Ia to the donor tissue did not occur either, suggesting that the lack of Ia -binding is due to the absence of an immune response and not to a direct interaction between the immunosuppressive drugs and the endothelial cells. The absence of binding of anti- Ia in these allografts was confirmed by immunofluorescence; on day 62 after transplantation on nude recipients, no positive fluorescence staining was observed on donor endothelial cells.

We have also examined anti- Ia binding after withdrawal of immunosuppressive treatment. In this experiment, B10.T(6R) recipients of B10.AQR skin grafts received daily CyA and the uptake of monoclonal Ia antibody by the graft was assessed at different days after transplantation. In five mice the CyA administration was discontinued on day 14. This leads to rejection of the skin grafts within 10–13 days¹⁴. The results show that withdrawal of the immunosuppressive agent resulted in uptake of radiolabel within 3 days. In the group that received CyA throughout, binding was not detectable at any time (Fig. 3).

Our results show that donor type Ia antigens are present on vascular endothelium of skin allografts in the mouse. Their expression increases at the onset of rejection, whereas T-cell deficiency or administration of immunosuppressive agents suppresses it. Subsequent withdrawal of immunosuppression results in reappearance of the Ia antigens. A variable expression of Ia antigens has also been found on mouse peritoneal macrophages^{15–17}. Ia expression on these cells is regulated by a lymphokine elaborated by activated T cells¹⁸ which is similar to immune interferon¹⁹.

It seems likely that the expression of Ia antigens on vascular endothelium in our system is also under the influence of activated T cells. This would explain why endothelial Ia antigens have never been found in normal mouse skin apart from on epidermal Langerhans cells. We did not observe binding to cells in the epidermis. It is, however, possible that these cells could not be reached by sufficient amounts of antibody to be detectable by autoradiography. After administration of high

Table 1 Binding of radioiodinated anti- Ia^k monoclonal antibodies to mouse skin allografts at day 7 after transplantation

Donor	Recipient	H-2 haplotype	No. mice	Treatment	Binding $\pm$ s.d. (c.p.m.)
B10.BR	BALB/c	k $\rightarrow$ d	5	—	2,900 $\pm$ 750
B10.D2	BALB/c	d $\rightarrow$ d	5	—	350 $\pm$ 100
B10.T(6R)	B10.D2	y2 $\rightarrow$ d	6	—	510 $\pm$ 80
B10.BR	B10.D2	k $\rightarrow$ d	10	—	2,500 $\pm$ 600
B10.BR	B10.D2	k $\rightarrow$ d	10	RAMLS*	330 $\pm$ 90
B10.AQR	B10.T(6R)	y1 $\rightarrow$ y2	6	—	2,038 $\pm$ 650
B10.AQR	B10.T(6R)	y1 $\rightarrow$ y2	5	Cyclosporin A [†]	370 $\pm$ 10
B10.BR	BALB/c <i>nu/nu</i>	k $\rightarrow$ d	8	—	450 $\pm$ 100
B10.D2	BALB/c <i>nu/nu</i>	d $\rightarrow$ d	5	—	390 $\pm$ 150

* Rabbit-anti-mouse lymphocyte serum (0.25 ml) was injected intraperitoneally at days 0, 2 and 4 after transplantation.

[†] Cyclosporin A (Sandoz) was dissolved in olive oil at a concentration of 20 mg ml^{-1} and administered orally (75 mg per kg , daily).

doses of antibody, labelled with biotin, we have observed occasional binding to basal keratinocytes.

Our results suggest that, in skin grafting across Ia incompatibilities, where Ia antigens on passenger cells initiate the immune response, the same antigens present on vascular endothelium are the primary targets for the rejection process. This latter assumption is supported by a study in allografts of human skin, which showed that microvascular damage invariably preceded epithelial necrosis²⁰. This could explain the contradictory results obtained with skin from bone marrow chimaera grafted to Ia-incompatible recipients²¹. If syngeneic skin, repopulated with allogeneic *I*-region incompatible passenger cells is transplanted, rejection does not occur. This

could be because in these skin grafts allogeneic Ia-positive endothelial cells are not available as targets. On the other hand, allogeneic skin grafts repopulated with cells syngeneic to the recipient are also often not rejected in *I*-region incompatible combinations. Under these circumstances the endothelial Ia antigens will not be expressed, because the syngeneic passenger cells fail to stimulate the immune response of the recipient.

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1. Tamaki, K., Stingl, G., Gullino, M., Sachs, D. H. & Katz, S. I. *J. Immun.* **123**, 784-787 (1979).
2. Rowden, G., Phillips, T. M. & Delovitch, T. L. *Immunogenetics* **7**, 465-471 (1978).
3. Hirschberg, H., Moen, T. & Thorsby, E. *Transplantation* **28**, 116-121 (1979).
4. Hayry, P., von Willebrand, E. & Anderson, L. C. *Scand. J. Immun.* **11**, 303-310 (1980).
5. Natali, P. G., De Martino, C., Marcellini, M., Quaranta, V. & Ferrone, S. *Clin. Immun. Immunopath.* **20**, 11-20 (1981).
6. Baldwin III, W. M., Claas, F. H. J., van Es, L. A. & van Rood, J. J. *Transplant Proc.* **13**, 103-107 (1981).
7. Lampert, I. A., Sutters, A. J. & Chisholm, P. M. *Nature* **293**, 149-150 (1981).
8. Mason, D. W., Dallman, M. & Barclay, A. N. *Nature* **293**, 150-151.
9. Sutters, A. J. & Lampert, I. A. *Br. J. exp. Path.* **63**, 207-213 (1982).

10. Hunter, W. M. & Greenwood, P. C. *Nature* **194**, 459-460 (1962).
11. Hoyer, L. W., De Los Santos, R. P. & Hoyer, J. R. *J. clin. Invest.* **52**, 2737-2744 (1973).
12. Bowman, P. D. *et al. In Vitro* **17**, 353-362 (1981).
13. Ozato, K., Mayer, N., & Sachs, D. H. *J. Immun.* **124**, 533-540 (1980).
14. Lems, S. P. M. & Koene, R. A. P. *ICRS med. Sci.* **7**, 184 (1979).
15. Beller, D. I. & Ho, K. J. *J. Immun.* **129**, 123-131 (1982).
16. Steeg, P. S., Moore, R. N. & Oppenheim, J. J. *J. exp. Med.* **152**, 1734-1744 (1980).
17. Steinman, R. N., Nogueira, N., Witmer, M. D., Tydings, J. & Mellman, I. S. *J. exp. Med.* **152**, 1248-1261 (1980).
18. Scher, M. G., Beller, D. I. & Unanue, E. R. *J. exp. Med.* **152**, 1684-1698 (1980).
19. Steeg, P. S., Johnson, H. M. & Oppenheim, J. J. *J. Immun.* **129**, 2402-2406 (1982).
20. Dvorak, H. F. *et al. J. exp. Med.* **150**, 322-337 (1979).
21. Woodward, J. G., Shigekawa, B. L. & Frelinger, J. A. *Transplantation* **33**, 254-259 (1982).

***Plasmodium falciparum* strain-specific antibody blocks binding of infected erythrocytes to amelanotic melanoma cells**

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An important feature of *Plasmodium falciparum* malaria which differentiates it from other human malarias is that erythrocytes infected with trophozoites and schizonts are not present in the peripheral blood but are sequestered along capillary and venular endothelium¹. Infected erythrocytes attach via parasite-induced ultrastructural modifications on the surface of the infected cells, called 'knobs'^{2,3}. This sequestration may be important for parasite survival because it prevents infected erythrocytes from circulating through the spleen where they could be eliminated. We have established an *in vitro* correlate of sequestration and used it to demonstrate that immune sera from repeatedly infected *Aotus* monkeys inhibit binding of infected erythrocytes to endothelial cells⁴. We have investigated whether antiserum that blocks binding of one isolate of *P. falciparum* to target cells can block or reverse binding of other isolates. We report here that sera which block or reverse binding are strain-specific, indicating that the corresponding antigens on the surface of the infected erythrocytes are strain (isolate)-specific.

We used amelanotic melanoma cells instead of endothelial cells as targets for binding of the infected erythrocytes. Melanoma cells bind infected erythrocytes by the same mechanism as endothelial cells⁵. Furthermore, the level of inhibition of binding of erythrocytes infected with the Camp isolate of *P. falciparum* by anti-Camp serum at various dilutions was similar for endothelial and melanoma cells (data not shown).

Isolates of *P. falciparum* from three different areas of the world were used to immunize *Aotus* monkeys (see Table 1 for details). Sera at a 1:2 dilution were tested for inhibition of binding of erythrocytes infected with homologous isolates; the three sera consistently showed >90% inhibition of binding. To determine whether the blocking antibodies in the three antisera cross-reacted with an erythrocyte surface antigen common to the three isolates, the sera were preabsorbed extensively with homologous- or heterologous-infected erythrocytes to remove antibodies to corresponding targets on the surface of the infected cells. The preabsorbed sera were then tested for inhibition of binding of homologous isolates (Table 1). Preabsorption totally abolished or markedly reduced blocking activity for the homologous-infected erythrocyte; preabsorption with heterologous isolates resulted in little or no reduction in the blocking activity. Thus both the blocking antibodies and their targets on the surface of infected erythrocytes seemed to be specific for each isolate.

We observed that aggregates of infected erythrocytes formed during the binding assay and these aggregates seemed to increase in number and size after incubation with immune serum. Thus, it was possible that the blockade of binding might in part have resulted from agglutination of infected erythrocytes by immune serum. Therefore, we added the immune sera at a 1:2 dilution after the infected cells had become bound to the melanoma cells to determine whether the antibody would reverse binding. The three immune sera reversed binding of homologous-infected erythrocytes by >77% (Table 2). Anti-Camp serum did not reverse binding of the other two isolates, whereas the anti-St Lucia serum did reverse binding of the FMG isolate. At dilutions of 1:2 and 1:4, anti-St Lucia serum reversed binding of the St Lucia isolate by 90% and 23% and of the FMG isolate by 76% and 45%, respectively (Table 2). Cross-reaction of anti-FMG serum with the St Lucia isolate was minimal.

We tested the effect of human immune sera on binding of infected human erythrocytes, using 12 sera from adults in The Gambia and Sudan and *P. falciparum* isolates from Brazil and Vietnam. Of the 12 sera, 6 consistently inhibited binding of both isolates by >80%. Two sera which inhibited binding of the Brazilian parasite by about 90% had only minimal effect on the Vietnam isolate. Two had little effect on either isolate. When three of the sera which inhibited binding of both isolates were absorbed with infected erythrocytes, the inhibitory activity

Table 1 Effect of preabsorption of antisera with various strains of infected erythrocytes (IRBCs) on their ability to inhibit binding

Combinations of sera and IRBCs used in binding assay		Per cent inhibition of binding after absorbing sera with:				
Sera	IRBCs	No preabsorption	Normal <i>Aotus</i> RBCs	Camp IRBCs	FMG IRBCs	St Lucia IRBCs
Anti-Camp	Camp	100	96	6	96	100
Anti-FMG	FMG	100	100	99	36	97
Anti-St Lucia	St Lucia	96	96	70	100	0

Sera raised against the three isolates of *P. falciparum*: Camp HQ from Malaysia, FMG(FCR-3) from The Gambia and St Lucia from El Salvador, were obtained by immunizing three different *Aotus* monkeys. The monkeys (*Aotus trivirgatus griseomembra*) were immunized by repeated injection with infected erythrocytes and drug-cured, with 2–4 weeks between infections. Control sera were withdrawn from the monkeys before infection. The monkeys were considered immune when a challenge with 2×10^7 to 2×10^9 infected erythrocytes of the homologous isolate failed to produce any detectable parasitaemia. Usually the monkeys were immune after 3–4 infections. Immune sera were withdrawn at various times after demonstration of immunity. Erythrocytes infected with young parasites (rings) were obtained from non-immune *Aotus* monkeys and cryopreserved at 15–20% parasitaemia and thawed and cultured to trophozoites and schizonts when required for binding assay, as described previously¹⁴. Absorption of serum was achieved by incubating with an equal volume of packed infected and uninfected erythrocytes for 30 min in a 37 °C water bath. The normal, uninfected erythrocytes were obtained from two monkeys before infection with the Camp and FMG isolates used for absorption. The combined normal erythrocytes were cryopreserved and later thawed when needed for absorption. To determine the effect of the serum on binding, the test serum was incubated with an equal volume of packed infected cells (10–20% parasitaemia) at 37 °C for 30 min. RPMI 1640, pH 7.4, containing 25 mM sodium bicarbonate was then added to bring the haematocrit of the cell suspension to 2–4%; the suspension was then laid over melanoma cells which had been fixed for 1 h with 10% phosphate-buffered formalin (Fisher) diluted to 1% with phosphate-buffered saline, pH 7.3, then washed with RPMI 1640. The binding of infected erythrocytes to fixed melanoma cells and endothelial cells was identical to binding to unfixed cells (I.J.U., unpublished data). Binding of the infected erythrocytes to the target cells was assayed as described previously^{4,5} and the number of infected erythrocytes attached per target cell (IRBCs per TC) was calculated. In the presence of normal *Aotus* serum, 16 ± 6 , 3.2 ± 0.7 and 22 ± 8 erythrocytes infected with Camp, FMG and St Lucia isolates, respectively, were attached per target cell. The effect of the immune sera on binding was determined by comparing the number of infected cells bound to target cells in the presence of control serum and that bound in the presence of the test serum. The per cent inhibition of binding (PI) was calculated as follows:

$$PI = \frac{\text{IRBCs per TC in presence of control serum} - \text{IRBCs per TC in presence of test serum}}{\text{IRBCs per TC in presence of control serum}} \times 100.$$

Table 2 Reversal of binding of IRBCs by immune sera raised against homologous and heterologous *P. falciparum* isolates

		Per cent reversal of binding (mean $\pm$ s.e.)		
Sera	Dilution	Camp IRBCs	FMG IRBCs	St Lucia IRBCs
Anti-Camp	1:2	77 $\pm$ 5	2 $\pm$ 11	2 $\pm$ 2
	1:4	53 $\pm$ 12	–4 $\pm$ 11	–6 $\pm$ 8
Anti-FMG	1:2	23 $\pm$ 7	99 $\pm$ 0.6	39 $\pm$ 3
	1:4	–1 $\pm$ 9	99 $\pm$ 1	–13 $\pm$ 7
Anti-St Lucia	1:2	7 $\pm$ 20	76 $\pm$ 9	90 $\pm$ 4
	1:4	–4 $\pm$ 10	45 $\pm$ 15	23 $\pm$ 14

Sera obtained before infection (control sera), immune sera (test sera) and infected erythrocytes were obtained as described in Table 1 legend using different monkeys for the immune sera and infected erythrocytes. To determine the reversal effect of the sera, infected cells were allowed to bind to the target cells in an 8-well Lux plate (Flow) with an area of 7.5 cm² per well. After washing out unbound cells, the test serum at a 1:2 or 1:4 dilution with RPMI-1640 containing 25 mM HEPES and 24 mM NaHCO₃, pH 7.4, was added to the bound cells; 0.5 ml of the diluted serum was added per well so that the fluid covered the bottom of the test plate. The cells plus serum were then incubated at 37 °C for 30 min with gentle swirling every 15 min. After incubation, the detached erythrocytes were washed out and those still attached were counted either visually after fixing with 2% glutaraldehyde and staining with Giemsa⁴, or by radioactivity using infected cells metabolically labelled with radioactive hypoxanthine. The data shown are the mean and standard error of three results: two were obtained by radioactivity measurements and one by counting of stained cells. In the presence of control *Aotus* serum, 30, 41 and 13 erythrocytes infected with Camp, FMG and St Lucia isolates, respectively, were attached per target cell. Similar results were obtained by radioactivity measurements and by counting. Therefore they were combined. Results showing the same specificity as above were obtained using sera and infected erythrocytes from a different set of monkeys. Per cent reversal of binding was calculated by using the following formula:

$$\text{per cent reversal} = \frac{\text{control serum values} - \text{test serum values}}{\text{control values}} \times 100.$$

was abolished or markedly reduced for the isolate used for absorption, with little or no effect on the inhibition of the other isolate (Table 3). Thus, inhibitory activity in serum seems to be strain-specific although immune sera from adults in the endemic areas may contain a mixture of antibodies of different specificities.

The inhibition of binding of both monkey and human sera was independent of complement and was mediated by IgG eluted from protein A–Sepharose. IgM had no effect.

Strain-specific variation of antigenic determinants, especially for those antigens under immune attack, may be a mechanism for parasite survival. Strain-specific antigens in *P. falciparum* have been studied by others using different approaches; most have examined antigens other than those on the surface of infected erythrocytes. For example, strain specificity in *P. falciparum* was described for soluble antigens in the plasma^{6,7} and for antigens on the surface of merozoites⁸ and mature parasites⁹.

In a recent study using indirect immunofluorescence¹⁰, Hommel *et al.* suggested the possibility of intrastrain and interstrain variation of antigens on the surface of *P. falciparum*-infected erythrocytes. We have shown here that indeed there are strain-specific antigens on the *P. falciparum*-infected erythrocyte surface. Our studies are relevant to mechanisms by which the parasite might avoid the host immune response. By sequestering along the venules, infected erythrocytes avoid the powerful immune forces in the spleen. The present study suggests that the host produces antibody that blocks this binding. In addition, we have observed that immune sera that reversed binding to melanoma cells *in vitro* would release sequestered parasites *in vivo* in squirrel monkeys¹¹. The strain specificity in antibody that blocks sequestration *in vitro* might explain, in part, the strain specificity of acquired protection against *P. falciparum* infection in man¹². The multiplicity of specificities of the antibodies in sera from adults in endemic areas suggests the existence of multiple strains of *P. falciparum* in these areas; thus acquired resistance against malaria may depend on exposure to and immunity against the different strains. As sequestration of infected erythrocytes in cerebral blood vessels has been implicated in the production of cerebral malaria,

Table 3 Effect of preabsorption of human antisera with various strains of IRBCs on their ability to inhibit binding

Combinations of sera and IRBCs used in binding assay		Per cent inhibition of binding after absorbing sera with:			
Sera	IRBCs	No preabsorption	Normal human RBCs	Vietnam (V1) IRBCs	Brazil (ItG2F6) IRBCs
G725	ItG2F6	93 ± 1	80.5 ± 2.5	84.5 ± 3.5	0 ± 0
	V1	92.5 ± 0.5	75 ± 6	33 ± 0	84.5 ± 5.5
S1713	ItG2F6	94.5 ± 0.5	95 ± 1	79 ± 4.0	28.5 ± 9.5
	V1	86 ± 2	90 ± 2	30 ± 0.5	91.5 ± 2.5
S1877	ItG2F6	92.5 ± 1.5	94 ± 0	94 ± 0	34 ± 10.5
	V1	71 ± 13.5	44	0	56

Sera were obtained from healthy adult volunteers without malarial symptoms in The Gambia (G72S) and Sudan (S1713 and S1877) and stored at -80 °C. *P. falciparum* isolates obtained from Brazil (ItG2F6) and Vietnam (V1) were maintained in continuous cultures¹⁴. Absorption of serum was achieved by incubation for 30 min at 37 °C with an equal volume of packed infected erythrocytes or uninfected O Rh⁻ erythrocytes, the same erythrocytes used in culture. Parasitaemia of infected erythrocytes used for absorption and binding tests was 5–10%. Each serum aliquot was absorbed twice and tested for inhibition of binding as described in Table 1. The data are the mean of two experiments except S1877, V1, which is one experiment. In the presence of normal AB serum from adults in the United States, 4.2 ± 0.7 and 6.5 ± 1.3 erythrocytes infected with ItG2F6 and V1 isolates, respectively, were bound per melanoma cell.

reversal or blockade of cerebral sequestration by the correct strain-specific antibody may reverse or prevent this clinical complication.

This study has established the existence of strain specificity of antigenic determinants on the surface of *P. falciparum*-infected erythrocytes, and has demonstrated the existence of cross-reacting antigens. Indeed, in another study using immune electron microscopy, evidence was presented that cross-reacting antigens may exist over knobs¹³. We now need biochemically to characterize the malarial antigens on the infected erythrocyte surface including components necessary for binding to endothelium, to determine whether there are common exposed epitopes among different isolates.

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1. Miller, L. H. *Am. J. trop. Med. Hyg.* **18**, 860–965 (1969).
2. Rudzinska, M. A. & Trager, W. *J. Protozool.* **15**, 73–88 (1968).
3. Luse, S. A. & Miller, L. H. *Am. J. trop. Med. Hyg.* **20**, 655–660 (1971).
4. Udeinya, I. J., Schmidt, J. A., Aikawa, M., Miller, L. H. & Green, I. *Science* **212**, 555–557 (1981).
5. Schmidt, J. A. *et al. J. clin. Invest.* **70**, 379–386 (1982).
6. Wilson, R. J. M., McGregor, I. A., Hall, P., Williams, K. & Bartholomew, R. *Lancet* **ii**, 201–205 (1969).
7. Wilson, R. J. M. *Nature* **284**, 451–452 (1980).
8. Wilson, R. J. M. & Phillips, R. S. *Nature* **263**, 132–134 (1976).
9. McBride, J. S., Walliker, D. & Morgan, G. *Science* **217**, 254–257 (1982).
10. Hommel, M., David, P. H. & Oligino, L. *J. exp. Med.* **157**, 1137–1148 (1983).
11. David, P. H., Hommel, M., Miller, L. H., Udeinya, I. J. & Oligino, L. D. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
12. James, S. P., Nicol, W. D. & Shute, P. G. *Proc. R. Soc. Med.* **25**, 1153–1186 (1932).
13. Langreth, S. & Reese, R. T. *J. exp. Med.* **150**, 1241–1254 (1979).
14. Trager, W. & Jensen, J. B. *Science* **193**, 673–675 (1976).

Androgen regulation of cell proliferation and expression of viral sequences in mouse mammary tumour cells

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The role of steroids in promoting cell proliferation is well established^{1–3} but the molecular mechanisms are not clear. The S115 mouse mammary tumour cell line provides a model system for molecular studies *in vitro* in that it exhibits in tissue culture both a positive proliferative response to androgens and a change from a transformed phenotype in the presence of androgen to a normal phenotype when androgen is removed. We have considered here the possible involvement of mouse mammary tumour virus (MMTV) in these processes. We have demonstrated the presence in S115 cells of MMTV-related sequences which are transcribed into RNA only in the long-term presence of androgen. Prolonged culture in the absence of androgen, which results in loss of proliferative response to androgen, is accompanied by loss of MMTV-related RNA and increased methylation of MMTV-related sequences.

The S115 cell line was derived from the androgen-dependent Shionogi 115 mouse mammary carcinoma⁴ and exhibits a proliferative response to androgens when cultured in the presence of testosterone (A⁺ cells)⁵. After several weeks of culture in the absence of testosterone, however, the cells become unre-

sponsive to androgens (A⁻ cells)^{6,7}. This loss of response is accompanied by a change from a fibroblastic to an epithelial morphology^{8,9}, increased sensitivity to various growth stimuli⁷, a reduction in the rate of cell growth¹⁰, an increased density regulation^{7,11}, much greater dependence on attachment to the substrate^{10,12}, an alteration in cytoskeletal structure¹³, and lost ability to grow in suspension cultures⁹. As both cell types give rise to tumours when injected into male nude mice⁹, these alterations in phenotype in culture cannot be related directly to tumorigenesis *in vivo*. However, as judged by criteria of cell phenotype *in vitro*, androgens do seem to control a change from a normal (A⁻ cells) to a transformed (A⁺ cells) phenotype in this cell line which is related to the proliferative response. In view of the well documented involvement of MMTV in the development of mammary carcinomas in the mouse, we considered it possible that viral elements might have some role in modulating the phenotype of the S115 cell line.

Most inbred strains of mice contain endogenous MMTV sequences, stably inherited through the germ line¹⁴. Although they seem to be complete proviral units, they may or may not be expressed, depending presumably on their location within the host DNA. However, tumorigenesis correlates usually with the acquisition of additional proviral elements, possibly in defined regions of the chromosome (ref. 15 and G.P. and C.D., unpublished results). Proviral MMTV DNA contains at least three genes, *gag*, *pol* and *env*, bounded at both ends by long terminal repeat (LTR) segments^{16,17} (Fig. 1). Expression of the provirus is stimulated by glucocorticoids^{18–20}, but not oestrogens or androgens²¹ and more recent studies have located the site of glucocorticoid action to within the LTR region^{22–24}.

The characterization of MMTV sequences in the S115 cell line has relied on restriction enzyme digestion and Southern blotting. The restriction enzyme *EcoRI* cleaves MMTV proviral

DNA once (Fig. 1), creating a 5' segment containing LTR and *gag-pol* sequences and a 3' segment containing LTR and *env* sequences. Using probes specific for the 5' (*gag-pol*) or 3' (*env*) half of the virus or for the LTR (Fig. 1), it can be seen that S115 cells contain approximately three MMTV proviruses (Fig. 2, tracks 3–6). In the absence of control mouse tissue, it was not possible to distinguish between endogenous or newly acquired viruses. However, this was not considered important in this study as the MMTV sequences were exploited as a set of marker genes whose status might be influenced by androgens.

Loss of androgen response associated with the change from A^+ to A^- phenotype was not accompanied by any obvious changes in number or position of the MMTV-LTR-related sequences (Fig. 2, tracks 1–6). However, distinct differences were observed in the extent of methylation of these DNA sequences, using the isoschizomeric restriction enzymes *MspI* and *HpaII*. These enzymes cut unmethylated DNA at the same sites but differ in their sensitivity to cytosine methylation²⁵. By these criteria, sites near or within the LTR regions of S115 DNA seemed to be more extensively methylated in the androgen unresponsive A^- DNA than in the androgen responsive A^+ DNA (Fig. 2, tracks 7–12).

S115 cells were studied further to determine whether these MMTV-related sequences were expressed in an androgen-sensitive way. Androgen-stimulated (A^+) cells produced at least four RNA species homologous to a MMTV-LTR probe (Fig. 3, track 2). The major RNA species was about 16S although small amounts of the viral 35S, 24S and 20S RNAs were found also. This contrasted to the pattern of RNA species present in MMTV-infected mink cells where the 16S RNA was barely detectable (Fig. 3, track 1)^{26,27}. In contrast, these RNAs were undetectable in A^- cells in three separate experiments, one of which is shown in Fig. 3, track 4. These MMTV-LTR-related RNAs were all polyadenylated (Fig. 3, track 13) and therefore unlikely to represent nonspecific hybridization to rRNA. Furthermore, it is known that androgens do not alter gross RNA levels per S115 cell²⁸, confirming that the modulation of MMTV RNA levels was a specific effect. Hybridization using probes specific to various regions of the MMTV genome (Fig. 3) confirmed that the 35S RNA included *gag-pol* (track 15), *env* (track 16) and LTR (track 14) sequences; the 24S RNA contained *env* (track 16) and LTR (track 14) sequences; whereas the 16S RNA was only detected with the LTR probe (track 14) and U_3 -specific probe (data not shown), although weak hybridization to *env* (track 16) sequences was also observed. The fact that this 16S RNA hybridizes to the LTR and U_3 probes and weakly to *env* suggests that it could encode the putative 'orf' protein^{17,29}.

A time course of the loss of these RNAs from A^+ cells during the period of androgen withdrawal and loss of androgen growth responsiveness, revealed loss of RNA after 6 days (Fig. 3, track 11). The use of dextran-charcoal to remove endogenous steroids in fetal calf serum (DC-FCS) produced a more rapid effect (track 11) than unstripped fetal calf serum (FCS) (tracks

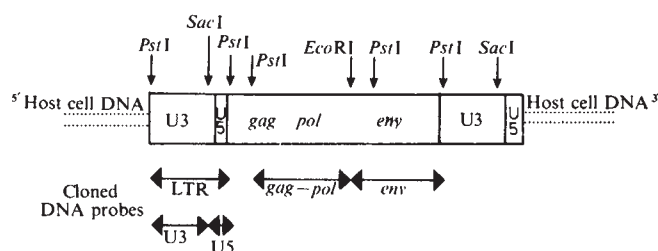


Fig. 1 Restriction map of MMTV proviral DNA (milk-borne strain from GR mice) to show the specific DNA probes used in these experiments. The following fragments of the MMTV provirus were cloned into plasmid pAT 153: MMTV-LTR contains the 1.4-kilobase (kb) *PstI* fragment, MMTV-*env* the 2.7-kb *EcoRI*-*PstI* partial digest fragment, MMTV-*gag-pol* the 3.4-kb *PstI*-*EcoRI* fragment, MMTV- U_3 the 1.1-kb *PstI*-*SacI* fragment.

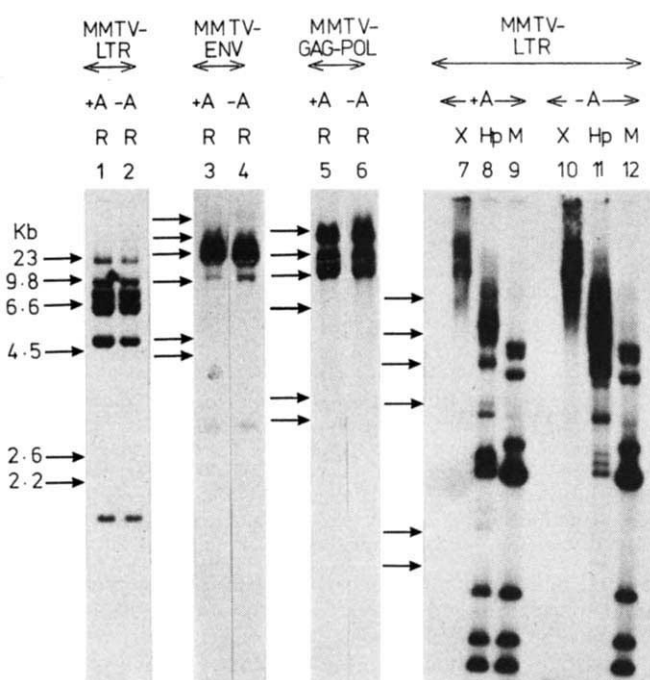


Fig. 2 Restriction endonuclease analysis of MMTV-related sequences in the DNA of S115 cells. DNA from either A^+ or A^- cells was either undigested (X) or digested with *EcoRI* (R), *HpaII* (Hp) or *MspI* (M). ^{32}P -labelled probes were used as indicated and as described in Fig. 1 legend. Molecular weight standards were provided by a *HindIII* digest of bacteriophage λ DNA and their positions are shown by arrows.

Methods: S115 cells were grown as monolayer cultures in the long-term presence (+A) or absence (-A) of testosterone as described previously⁷. High-molecular weight DNA was prepared from the cells^{36,37}, and was digested with restriction enzymes. The fragments were separated on agarose gels (10 μ g DNA per track), transferred to nitrocellulose³⁸ and hybridized³⁹ to 5×10^6 c.p.m. of ^{32}P -labelled probes^{40,41}. Hybridization was in $1 \times$ SSC, $10 \times$ Denhardt's, 0.1% SDS, 50 μ g ml⁻¹ single-stranded calf thymus DNA and 10% dextran sulphate at 65 °C for 16 h. Blots were washed at a stringency of $0.2 \times$ SSC, 0.1% SDS for 1 h at 65 °C. The cloned DNA fragments were isolated from the plasmid⁴¹ before ^{32}P -labelling by nick-translation.

6–8). This effect on RNA was androgen-specific and not due to removal of other growth factors by dextran-charcoal because A^+ cells grown in the presence of added testosterone gave similar levels of RNA in both FCS and DC-FCS (data not shown). The loss of LTR-related RNA in FCS follows a similar time course to the loss of androgen responsiveness of proliferation¹⁰. In contrast, restoration of androgen responsiveness to A^- cells is slower and may depend on how long the cells have been grown without testosterone (unpublished data). However, in accordance with the cell biology, the recovery of MMTV-specific RNA levels was also slower during this period (Fig. 3, track 12).

As the synthetic glucocorticoid, dexamethasone, is known to stimulate transcription of the MMTV provirus, we studied how it might interact with androgens to affect the levels of MMTV-LTR-related RNAs in S115 cells. Dexamethasone did not alter the types of RNA present but it did result in an increase in LTR-related RNAs in A^+ cells deprived of testosterone for 4 weeks (Fig. 3, track 10). However, even in the presence of dexamethasone, no RNA could be detected in the A^- cells (Fig. 3, track 5).

The implications of our observations are relevant to several areas of steroid hormone action. Although we have no direct proof that the androgen-modulated expression of viral RNA is causally related to the proliferative response, the data presented here suggest an association between the two and also an involvement of DNA methylation in the process. DNA methyl-

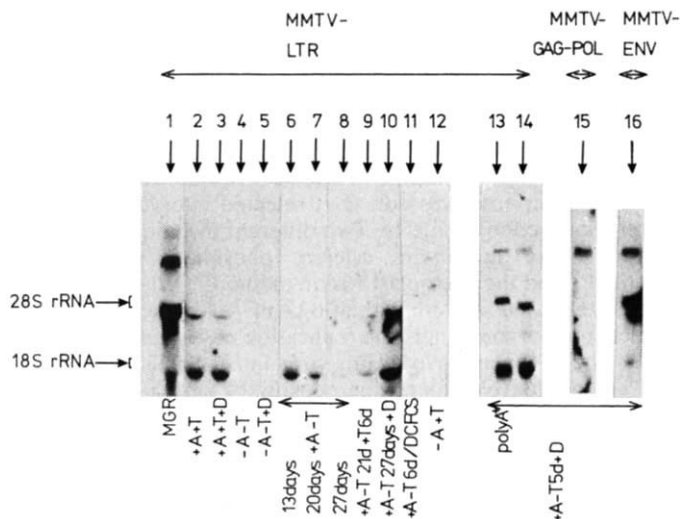


Fig. 3 Analysis of MMTV-related RNA of S115 cells including the effects of androgen and dexamethasone. Track 1, MMTV-LTR-related RNA in MGR cells (mink cells infected with the GR strain of MMTV); tracks 2–16, S115 cells. S115 cells were grown in the following conditions: A⁺ cells +T (2), A⁺ cells +T +D (3), A⁻ cells -T (4), A⁻ cells -T +D (5), A⁺ cells -T 13 days (6), A⁺ cells -T 20 days (7), A⁻ cells -T 27 days (8), A⁺ cells -T 21 days +T 6 days (9), A⁺ cells -T 27 days +D (10), A⁺ cells -T 6 days in DC-FCS (11), A⁻ cells +T 6 days (12), A⁺ cells -T 5 days +D (13–16). Track 14 has as a control 50 µg of total cytoplasmic RNA and track 13 has 5 µg of poly(A)⁺ RNA selected from the control sample. Tracks 15 and 16 show homology of the S115 RNA to the gag-pol and env regions of the MMTV genome.

Methods: S115 cells were grown as long-term cultures in the presence (+A) or absence (-A) of testosterone and as short-term cultures with (+T) or without (-T) testosterone and collected as described for Fig. 2. Preparation of DC-FCS has been described before⁴². Where indicated, dexamethasone (+D) was added at a concentration of 10⁻⁷ M 18 h before preparation of RNA. MGR cells were grown as the S115 cells in 5% FCS and dexamethasone was added for the final 18 h. Cytoplasmic RNA was prepared by resuspending the cell pellet in 0.1 M NaCl, 10 mM Tris, 1 mM EDTA, 0.5% Nonidet P40 pH 7.5 at 4 °C. The nuclear pellet was removed, the supernatant extracted with phenol-chloroform and the RNA precipitated in ethanol. Poly(A)⁺ RNA was isolated from total RNA by oligo(dT)-cellulose chromatography⁴³. RNA was analysed by electrophoresis (50 µg RNA per track except in track 13 which had 5 µg) in 1.5% agarose-formaldehyde gels⁴¹, blotted onto nitrocellulose and hybridized to the appropriate ³²P-labelled probe as indicated. Autoradiography was for 5 h (track 1), 18 h (tracks 2–12) or 24 h (tracks 13–16).

ation has been implicated in the control of MMTV expression³⁰ and in tumorigenesis in mice^{31–33} but evidence of its overall role in eukaryotic gene expression is conflicting^{34,35}. However, further work on the androgen control of these MMTV-related RNAs and the significance of DNA methylation therein may clarify the mechanism whereby tumour cells progress from steroid sensitivity to insensitivity.

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- King, R. J. B. *Biochem. Act. Horm.* **6**, 247–265 (1979).
- Sonnenschein, C. & Soto, A. M. *J. natn. Cancer Inst.* **64**, 211–215 (1980).
- Lippman, M. *Banbury Rep.* Vol. 8 (eds Pike, M. C., Siiteri, P. K. & Welsch, C. W.) 171–181 (Cold Spring Harbor Laboratory, 1981).
- Minesita, T. & Yamaguchi, K. *Cancer Res.* **25**, 1168–1175 (1965).
- Smith, J. A. & King, R. J. B. *Expl Cell Res.* **73**, 351–359 (1972).
- Stanley, E. R., Palmer, R. E. & Sohn, U. *Cell* **10**, 35–44 (1977).
- Yates, J. & King, R. J. B. *Cancer Res.* **38**, 4135–4137 (1978).
- Desmond, W. J., Wolbers, S. J. & Sato, G. *Cell* **8**, 79–86 (1976).
- Yates, J. & King, R. J. B. *Cancer Res.* **41**, 258–262 (1981).
- King, R. J. B., Cambray, G. J. & Robinson, J. H. *J. Steroid Biochem.* **7**, 869–873 (1976).

- Robinson, J. H. & Smith, J. A. *J. cell. Physiol.* **89**, 111–119 (1976).
- Yates, J., Couchman, J. R. & King, R. J. B. *Hormones and Cancer* (eds Iacobelli et al.) (Raven, New York, 1980).
- Couchman, J. R., Yates, J., King, R. J. B. & Badley, R. A. *Cancer Res.* **41**, 263–269 (1981).
- Traina, V. L., Taylor, B. A. & Cohen, J. C. *J. Virol.* **40**, 735–744 (1981).
- Nusse, R. & Varmus, H. E. *Cell* **31**, 99–109 (1982).
- Cohen, J. C., Majors, J. E. & Varmus, H. E. *J. Virol.* **32**, 483–496 (1979).
- Donehower, L. A., Huang, A. L. & Hager, G. L. *J. Virol.* **37**, 226–238 (1981).
- Parks, W. P., Ransom, J. C., Young, H. A. & Scolnick, E. M. *J. biol. Chem.* **250**, 3330–3336 (1975).
- Ringold, G. M., Yamamoto, K. R., Tomkins, G. M., Bishop, J. M. & Varmus, H. E. *Cell* **6**, 299–305 (1975).
- Ringold, G. M. *Biochim. biophys. Acta* **560**, 487–508 (1979).
- Young, H. A., Scolnick, E. M. & Parks, W. P. *J. biol. Chem.* **250**, 3337–3343 (1975).
- Hynes, N. E., Kennedy, N., Rahmsdorf, U. & Groner, B. *Proc. natn. Acad. Sci. U.S.A.* **78**, 2038–2042 (1981).
- Lee, F., Mulligan, R., Berg, P. & Ringold, G. *Nature* **294**, 228–232 (1981).
- Govindan, M. V., Spiess, E. & Majors, J. *Proc. natn. Acad. Sci. U.S.A.* **79**, 5157–5161 (1982).
- Waalwijk, C. & Flavell, R. A. *Nucleic Acids Res.* **5**, 4631–4641 (1978).
- Robertson, D. L. & Varmus, H. E. *J. Virol.* **30**, 576–589 (1979).
- Robertson, D. L. & Varmus, H. E. *J. Virol.* **40**, 673–682 (1981).
- Jagus, R. *Expl Cell Res.* **118**, 115–125 (1979).
- Dickson, C., Smith, R. & Peters, G. *Nature* **291**, 511–513 (1981).
- Cohen, J. C. *Cell* **19**, 653–662 (1980).
- Breznik, T. & Cohen, J. C. *Nature* **295**, 255–257 (1982).
- Drohan, W. N., Benado, L. E., Graham, D. E. & Smith, G. H. *J. Virol.* **43**, 876–884 (1982).
- Fanning, T. G., Vassos, A. B. & Cardiff, R. D. *J. Virol.* **41**, 1007–1013 (1982).
- Razin, A. & Riggs, A. D. *Science* **210**, 604–610 (1980).
- Felsenfeld, G. & McGhee, J. *Nature* **296**, 602–603 (1982).
- Pellicer, A., Wigler, M., Axel, R. & Silverstein, S. *Cell* **14**, 133–141 (1978).
- Wigler, M. et al. *Cell* **16**, 777–785 (1979).
- Southern, E. M. *J. molec. Biol.* **98**, 503–517 (1975).
- Wahl, G. M., Stern, M. & Stark, G. R. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3683–3687 (1979).
- Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. *J. molec. Biol.* **113**, 237–251 (1977).
- Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).
- Darbre, P. D., Yates, J., Curtis, S. & King, R. J. B. *Cancer Res.* **43**, 349–354 (1983).
- Aviv, H. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1408–1412 (1972).

Interferon inhibits transformation of mouse cells by exogenous cellular or viral genes

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Specific genes can be introduced into cultured mammalian cells by DNA transfer¹, sometimes to be stably integrated and expressed^{2,3}. Because interferon inhibits the transformation initiated by DNA viruses^{4–8}, we wondered if it might also affect transformation induced by recombinant plasmids and cellular genes. We show here that in mouse L cells interferon treatment prevents stable integration and expression of the transfected plasmids containing the cloned herpesvirus thymidine kinase (*tk*) gene, or the dihydrofolate reductase (*dhfr*) gene from hamster ovary cells⁹. In contrast, interferon does not prevent transient expression of the *tk* gene in unintegrated form.

In a first set of experiments, confirming a preliminary report¹⁰, we studied the effect of mouse interferon on the biochemical transformation of Ltk⁻ mouse cells by a recombinant plasmid (plasmid pAGO), carrying the cloned herpes simplex virus *tk* gene¹¹. The cells were transfected by the calcium phosphate precipitation technique and the *tk*⁺ colonies were selected as described in Fig. 1 legend. Interferon was added at various concentrations to the cell culture medium before or after the transfection. When the cells were pretreated with 50 U ml⁻¹ of interferon for 24 h before the transfection, a 14% inhibition in the number of *tk*⁺ colonies was observed. Using 1,000 U ml⁻¹, the *tk*⁺ colony formation was almost completely inhibited (98%). When the interferon treatment of the cells was initiated just after transfection and pursued for 2 weeks, the inhibition was 64% with 50 U ml⁻¹ and 100% with 500 U ml⁻¹. The same results were obtained when the cells were, in addition, pretreated with interferon for 24 h before transfection.

Several arguments favour the involvement of the homologous interferon molecule itself in this inhibitory process. Mock inter-

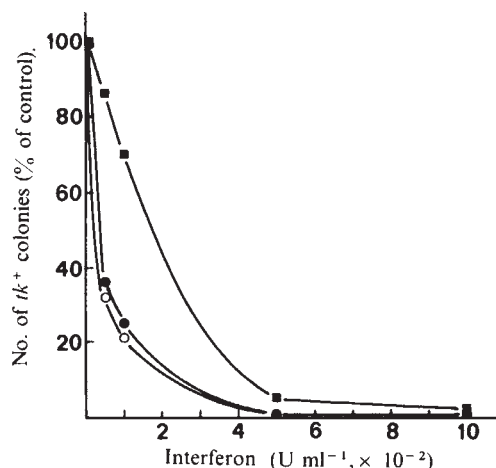


Fig. 1 Inhibition by interferon of *tk*-induced colony formation. Interferon was prepared on mouse EAT cells and titred 5×10^5 U ml $^{-1}$ with a specific activity of 10^6 U per mg of protein¹⁹. At the dilutions used here (500–10,000), no protein contaminant was as detected by electrophoresis on SDS-polyacrylamide gel. Ltk $^{-}$ aprt $^{-}$ cells (provided by R. Axel) were grown in Eagle's MEM containing 10% calf serum and antibiotics, in presence or absence of mouse interferon. Twenty-four hours later, subconfluent monolayers (25 cm 2 flasks; Corning) were transfected with 100 ng of plasmid pAGO 11 —plasmid pBR322 containing the cloned herpesvirus *tk* gene—by the calcium phosphate precipitation method¹² in the presence of 20 μ g of salmon sperm DNA as carrier. Interferon, at increasing concentrations, was added and maintained or not in the growth medium after transfection. HAT-selective medium²⁰ (hypoxanthine 15 μ g ml $^{-1}$, aminopterin 1 μ g ml $^{-1}$, thymidine 5 μ g ml $^{-1}$) was added 24 h later. The medium was changed every third day and the colonies were counted 2 weeks later. Colonies are expressed as the percentage of 71 colonies in untreated cells. Cells were treated by interferon: for 24 h before transfection (■), after transfection (●), and before and after transfection (○).

Table 1 Effect of the time of interferon treatment on the formation of *tk* $^{+}$ colonies in Ltk $^{-}$ cells transfected by plasmid pAGO

Time of interferon addition		No. of colonies per flask			
		Expt no.			
Before transfection	After transfection	1	2	3	4
—	—	28	10	17	45
18 h	—	0	7	1	8
3 h	—	0	11	5	29
—	1 h	0	0	0	0
—	1 day	0	0	2	4
—	2 days	0	0	6	10
—	3 days	0	0	NT	NT
—	4 days	5	0	9	NT
—	5 days	8	0	17	NT
—	6 days	14	0	18	10
—	7 days	18	5	14	30
—	8 days	24	7	17	40

Ltk $^{-}$ aprt $^{-}$ cells were grown in six-well dishes (Costar) and transfected either by the calcium phosphate precipitation method¹² (expt 1) or by the protoplast fusion technique of Sandri-Goldin *et al.*¹³ (expts 2–4). Protoplasts were obtained from *Escherichia coli* strain 1024 bacteria containing pAGO plasmid after amplification of the plasmid copy number by chloramphenicol. The protoplast solution (2 ml) was added to the cell monolayers (3×10^5 cells). The cell dishes were centrifuged at 2,000 r.p.m. for 20 min in an international PR $_2$ centrifuge using a swinging bucket rotor. The supernatant was removed by aspiration and 3 ml polyethylene glycol (50 g PEG per 50 ml minimal essential medium (MEM)) were added for 60 s. The cells were then rinsed three times and growth medium was added. Mouse interferon (500 U ml $^{-1}$) was added, or not, at different times before or after transfection. HAT-selective medium was added 24 h after transfection, and changed every 3 days with or without interferon.

feron produced no decrease in the number of *tk* $^{+}$ colonies. When neutralized by sheep anti-mouse interferon γ -globulin, interferon no longer had any effect on the genotypic transformation. Moreover, interferons from different species (rat, human α - and β -interferons) did not inhibit the transformation (data not shown).

To study the time course of its effect on the transformation process, interferon was added at selected intervals before or after transfection (Table 1). Two different techniques of transfection were used: the calcium phosphate precipitation method¹² and the protoplast fusion method¹³. When Ltk $^{-}$ cells were pretreated for 18 h with 500 U ml $^{-1}$ of interferon (a dose which inhibits completely the replication of vesicular stomatitis virus and encephalomyocarditis virus in Ltk $^{-}$ cells), only a few *tk* $^{+}$ colonies developed, compared with the untreated cells. The presence of interferon for only 3 h before transfection still had a strong inhibitory effect on the development of *tk* $^{+}$ colonies. When added on the day of the transfection, interferon completely inhibited colony formation. It is of interest that the number of *tk* $^{+}$ colonies was still significantly lower than that of controls when interferon treatment was initiated as late as 6 days after transfection. These observations, using two different transfer methods, make it unlikely that interferon treatment impairs the penetration of the recombinant plasmid in the cell. Moreover, because transformed *tk* $^{+}$ cells, as well as the *tk* $^{-}$ cells, can be maintained in the presence of interferon (500 U ml $^{-1}$) for many passages without significant effect on the rate of cell growth (data not shown), it may be assumed that interferon does not affect the expression of the *tk* gene once it is stably integrated into the cellular genome. To rule out the possibility that interferon inhibits the growth of *tk* $^{+}$ transfected cells in colonies in selective conditions, a few Ltk $^{-}$ cells were plated with an excess of *tk* $^{-}$ cells in hypoxanthine–aminopterin–thymidine (HAT)-selective medium. Whatever the number of *tk* $^{+}$ cells plated, there was no significant difference between interferon-treated cells or controls for the number of colonies observed in a range between 10 and 200 (data not shown).

To explore whether or not interferon's inhibitory effect on colony development was related to the viral nature of the *tk* gene, we tested its effect on transformation induced by cellular genes. Dominant mutant genes coding for drug resistance can be introduced in virtually any type of cell to which they thereafter confer drug resistance. For example, a Chinese hamster ovary cell line (A29) contains a mutant *dhfr* gene rendering these cells resistant to methotrexate (Mtx)⁹. The genomic DNA from these cells was used by Wigler *et al.*¹⁴ to transfer the mutant *dhfr* gene to Mtx-sensitive cells. When Ltk $^{-}$ cells were transfected with high molecular weight DNA extracted from this A29 cell line, an average of 10 Mtx-resistant colonies per flask developed, using 20 μ g of DNA (Table 2). Only one colony appeared in the presence of 500 U ml $^{-1}$ of interferon, whereas 70 colonies were seen in control cells. The same results were obtained after selection for the *tk* $^{+}$ colonies using high molecular weight cellular DNA from mouse L929 and Ltk $^{+}$ adenine phosphoribosyltransferase negative (aprt $^{-}$) cells (data not shown).

Recent observations suggest that genes can be expressed transiently after transfer into recipient cells^{1,15}. This is the case for the *tk* gene, the expression of which is detected early after transfection by the incorporation of 3 H-thymidine into the cell nuclei¹⁶. It was of interest, therefore, to determine whether or not the phenotypic expression of exogenous genes was also affected by interferon. Using the protoplast fusion technique, we transferred the pAGO plasmid into Ltk $^{-}$ cells. Twenty-four hours after transfection, the cells were labelled with 3 H-thymidine and examined after autoradiography, as shown in Fig. 2. In untreated cells, an average of 40 strongly labelled nuclei out of 2×10^5 cells was detected per slide. Thus, the frequency of phenotypic expression of the *tk* gene was 5×10^{-3} , whereas that of genotypic transformation was about 10^{-4} . Surprisingly, in cells treated with interferon, as many as 400 nuclei

Table 2 Effect of interferon treatment on the number of methotrexate-resistant colonies appearing after transfection of Ltk⁻ cells with DNA from A29 cells

Expt	Interferon treatment	Mtx-resistant colonies/ no. of plates
1	-	6/1
	+	0/1
2	-	28/3
	+	0/3
3	-	36/3
	+	1/3

High molecular weight DNA was extracted from hamster A29 cells (provided by R. Axel) resistant to 40 $\mu\text{g ml}^{-1}$ Mtx⁹, as described by Wigler *et al.*¹⁴. Subconfluent monolayers of Ltk⁻ cells (25 cm² flasks, 10⁶ cells per flask) were transfected with 20 μg A29 DNA by the calcium phosphate precipitation method as described previously. The selective medium containing 0.1 $\mu\text{g ml}^{-1}$ Mtx was added 24 h later, changed every 3 days, and the colonies were counted after 3 weeks growth in selective medium.

were labelled with a frequency of 5×10^{-2} . Note that the treatment of cells by interferon increased 10-fold the frequency of the *tk* gene phenotypic expression, despite a complete inhibition of the transformation process.

Measuring the DNA uptake by mammalian cells with fluorescent dyes, Loyter *et al.*¹⁷ have shown that virtually all recipient cells take up DNA in the cytoplasm and that the crucial step is in fact the transport of the DNA from the cytoplasm to the nucleus. Moreover, Capecchi¹⁸ has reported that a pBR322/TK plasmid must be injected directly into the nucleus to be expressed at a high rate. This indicates that a transport of the DNA from the cytoplasm to the nucleus is a prerequisite for gene transcription and expression.

In our model, because interferon retains its inhibitory properties when added late after transfection, it seems unlikely that the DNA uptake is impaired. Furthermore, early expression of the *tk* gene is not inhibited by interferon but is, on the contrary, enhanced, as shown by ³H-thymidine incorporation into the nucleus of transfected cells. These results can be interpreted either by an increased uptake of DNA by the nuclei after interferon treatment or by a higher level of expression of the *tk* gene in the nuclei.

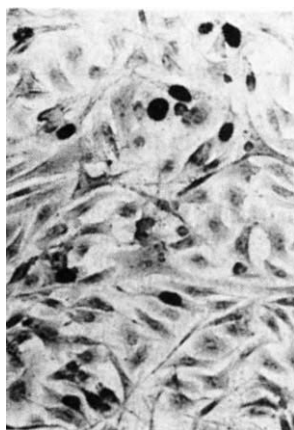


Fig. 2 Phenotypic expression of the *tk* gene. Ltk⁻ cells were grown on slides in 24-well dishes (Costar) and transfected by the protoplast fusion technique. Interferon (500 U ml⁻¹) was added, or not, to the medium after transfection. One day later, the cells were incubated for 24 h with ³H-thymidine (CEA, 25 $\mu\text{Ci ml}^{-1}$; specific activity 20 Ci mmol⁻¹) in MEM supplemented with 10% dialysed fetal bovine serum. Monolayers were fixed, autoradiographed, and photographed, as described by Linsley *et al.*¹⁶. The number of labelled nuclei was counted and compared with the number of cells present on the slide at the moment of transfection.

It is unclear why in interferon-treated cells this enhanced early expression of the *tk* gene is followed by an inhibition of the transformation. These unexpected observations might be explained either by the transient expression of the gene in an unintegrated form without subsequent integration, or by an abortive transformation.

On the basis of the data reported here, we stress that the inhibitory effect of interferon on gene integration in cellular DNA is not limited to viruses but is of more general nature. Thus, a physiological role of interferon could be the preservation or the regulation of established cellular genes.

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1. Pellicer, A. *et al. Science* **209**, 1414–1422 (1980).
2. Wigler, M. *et al. Cell* **11**, 223–232 (1977).
3. Wigler, M. *et al. Proc. natn. Acad. Sci. U.S.A.* **76**, 1373–1376 (1979).
4. Todaro, G. J. & Baron, S. *Proc. natn. Acad. Sci. U.S.A.* **54**, 752–756 (1965).
5. Todaro, G. J. & Green, H. *Proc. natn. Acad. Sci. U.S.A.* **55**, 302–308 (1966).
6. Taylor-Papadimitriou, J. & Stoker, M. *Nature new Biol.* **230**, 114–117 (1971).
7. Rapp, F. & Nishiyama, Y. *J. Virol.* **33**, 543–546 (1980).
8. Turek, L. P. *et al. UCLA Symp. molec. cell. Biol.* **25**, 181–191 (1982).
9. Flintoff, W. F., Davidson, S. V. & Siminovich, L. *Somatic Cell Genet.* **2**, 245–261 (1976).
10. Dubois, M. F., Vignal, M. & Chany, C. *C. r. heb. Séanc. Acad. Sci., Paris* **295**, 195–197 (1982).
11. Colbere-Garapin, F., Chousterman, S., Horodniceanu, F., Kourilski, P. & Garapin, A. C. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3755–3759 (1979).
12. Graham, F. L. & Van der Eb, A. J. *Virology* **52**, 456–467 (1973).
13. Sandri-Goldin, R. M., Goldin, A. L., Levine, M. & Glorioso, J. *Molec. cell. Biol.* **1**, 743–752 (1981).
14. Wigler, M. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 3567–3570 (1980).
15. Chang, L. J. A. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 146–150 (1982).
16. Linsley, P. S. & Siminovich, L. *Molec. cell. Biol.* **2**, 593–597 (1982).
17. Loyter, A., Scangos, G. A. & Ruddle, F. H. *Proc. natn. Acad. Sci. U.S.A.* **79**, 422–426 (1982).
18. Capecchi, M. R. *Cell* **22**, 479–488 (1980).
19. Dussaix, E., Grégoire, A., Chany, C., Thang, D. C. & Thang, M. N. *J. gen. Virol.* **64**, 285–290 (1983).
20. Szybalska, E. H. & Szybalski, W. *Proc. natn. Acad. Sci. U.S.A.* **48**, 2026–2034 (1952).

Polyoma virus transforming protein associates with the product of the *c-src* cellular gene

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Polyoma virus can transform the growth properties of rodent cells grown in culture and form tumours in susceptible animals¹, an activity largely due^{1–3} to one of the virus-encoded proteins, called middle T. Middle T has an associated tyrosine-specific protein kinase activity *in vitro*^{4–6} and interacts with cellular membranes⁷, but the biochemical basis of its ability to transform remains unclear. Although there is some correlation between the transforming activity of different polyoma virus mutants and their ability to accept phosphate on tyrosine in middle T in the *in vitro* kinase reaction⁴, the abundance of phosphotyrosine in protein is not elevated in polyoma virus-transformed cells⁸ and no cellular substrates for the putative kinase have been identified. It is also not yet known whether the tyrosine kinase of middle T is an intrinsic activity of the protein itself or the property of an associated enzyme. The experiments described here indicate that a fraction of middle T forms a stable complex with pp60^{c-src}, the product of a cellular oncogene⁹, and lead us to propose that the middle T associated kinase at least in part is a property of pp60^{c-src} rather than middle T itself.

The transforming protein of Rous sarcoma virus (RSV), called pp60^{v-src}, is better characterized than pp60^{c-src} and there

is good evidence that it has an intrinsic protein kinase activity that is directly involved in RSV transformation⁹. When the kinase activities of pp60^{v-src} or its cellular homologue pp60^{c-src} are assayed in immunoprecipitates of serum obtained from rabbits bearing RSV-induced tumours (TBR serum), phosphate is transferred to tyrosine in the heavy chain of TBR immunoglobulin⁹⁻¹². When extracts from polyoma virus-transformed cells are assayed in the same way using serum from animals bearing polyoma virus-induced tumours (anti-T serum), the transfer of phosphate to tyrosine in two alternative substrates has been observed. Using hamster anti-T serum, middle T becomes phosphorylated⁴, whereas using rat anti-T serum immunoglobulin molecules^{4,13} or middle T^{5,13} or both^{4,13} become phosphorylated.

Other tyrosine kinases capable of autophosphorylation can phosphorylate the heavy chain of the TBR antibody directed against pp60^{v-src} (refs 14, 15). We tested immunoprecipitates containing middle T for this activity. Immunoprecipitates of middle T were prepared, washed extensively and either normal rabbit or TBR serum added before the addition of [γ -³²P]ATP. Figure 1A shows that when extracts from polyoma virus-infected mouse 3T6 cells or from transformed rat cells were immunoprecipitated with hamster anti-T serum alone, middle T became phosphorylated (lanes 2, 6). When normal rabbit serum was added to the immunoprecipitates, middle T was still the only protein specifically phosphorylated (lanes 3, 7). However, when TBR serum was added before the kinase assay, both middle T and the TBR immunoglobulins became phosphorylated (lanes 4, 8). Separate experiments showed that label was associated with the heavy chain of TBR immunoglobulin

and that phosphorylation was on tyrosine residues (data not shown). When extracts from non-transformed Rat 1 cells (Fig. 1A, lanes 9–12) or uninfected 3T6 cells (data not shown) were used, no proteins were phosphorylated in the conditions used in Fig. 1.

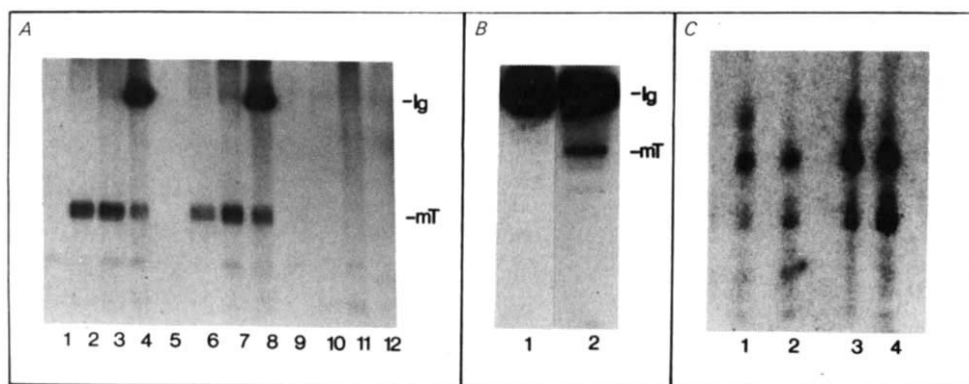
These results could mean that TBR immunoglobulin acted as a substrate for an intrinsic kinase activity of middle T. However, because the labelled TBR immunoglobulin was bound to the immunoprecipitate (data not shown), we concluded that the TBR serum recognized a protein present in the middle T immunoprecipitate and became phosphorylated in an immunocomplex. The antigen recognized by TBR serum could have been middle T or a cellular tyrosine kinase present in the immunoprecipitate. Because we knew that the TBR serum used could recognize rat and mouse pp60^{c-src}, whereas no cross-reactivity with middle T has been reported, we tested whether middle T and pp60^{c-src} co-immunoprecipitate. When TBR serum was used to immunoprecipitate pp60^{c-src} from untransformed cells and a kinase assay performed, the TBR immunoglobulin became phosphorylated (Fig. 1B, lane 1). When an immunoprecipitate from polyoma virus-transformed cells was assayed in the same way, two proteins were phosphorylated, one co-migrating with rabbit immunoglobulin, the other with middle T (Fig. 1B, lane 2). Partial proteolysis fingerprints (Fig. 1C) confirmed that this protein was indeed *in vitro* phosphorylated middle T. This result is consistent with the hypothesis that pp60^{c-src} and middle T form a stable complex with one another.

The phosphate acceptor activity of middle T assayed in the middle T associated kinase reaction reportedly sediments

Fig. 1 Kinase activities associated with middle T and pp60^{c-src}.

A: Lane 1, polyoma virus-infected 3T6 cells immunoprecipitated with normal hamster serum; lane 2, polyoma virus-infected 3T6 cells immunoprecipitated with hamster anti-T serum (prepared as described in ref. 4); lane 3, polyoma virus-infected 3T6 cells immunoprecipitated with hamster anti-T serum, then normal rabbit serum added before kinase assay; lane 4, polyoma virus-infected 3T6 cells immunoprecipitated with hamster anti-T serum, then TBR serum (prepared as described in ref. 19) added before kinase assay; lane 5, 2.8 cells immunoprecipitated with normal hamster serum; lane 6, 2.8 cells immunoprecipitated with hamster anti-T serum; lane 7, 2.8 cells immunoprecipitated with hamster anti-T serum, then normal rabbit serum added before assay; lane 8, 2.8 cells immunoprecipitated with hamster anti-T serum, then TBR serum added before kinase assay; lane 9, Rat 1 cells immunoprecipitated with normal hamster serum; lane 10, Rat 1 cells immunoprecipitated with hamster anti-T serum; lane 11, Rat 1 cells immunoprecipitated with hamster anti-T serum, then normal rabbit serum added before kinase assay; lane 12, Rat 1 cells immunoprecipitated with hamster anti-T serum, then TBR serum added before kinase assay. **B:** Lane 1, Rat 1 cells immunoprecipitated with TBR serum; lane 2, 2.8 cells immunoprecipitated with TBR serum. **C:** Lane 1, 55,000-MW protein from B, lane 1, 40 ng V8 protease; lane 2, 55,000-MW protein from B, lane 1, 200 ng V8 protease; lane 3, middle T from A, lane 2, 40 ng V8 protease; lane 4, middle T from A, lane 2, 200 ng V8 protease.

Methods: Rat 1 cells, 2.8 cells (a Rat 1 cell line transformed by a plasmid capable of expressing only middle T³) and polyoma virus-infected 3T6 cells were scraped from the dish in phosphate-buffered saline, then lysed in 120 mM NaCl, 50 mM Tris pH 8, 0.5% NP40 (buffer C). Insoluble material was removed by centrifugation at 4 °C for 5 min in an Eppendorf microfuge, and then supernatants used for immunoprecipitation as follows. Extracts were incubated with the appropriate antisera for 30 min at 4 °C, a two- to fourfold excess of fixed *S. aureus* added (previously boiled for 10 min, then washed 3 times in and resuspended in 150 mM NaCl, 51 mM EDTA, 50 mM Tris pH 7.5, 0.05% NP40 (NET.N)) and incubation continued for a further 30 min. Immunocomplexes were then collected by centrifugation for 45 s in a microfuge at 4 °C, then washed 3 times in 150 mM NaCl, 50 mM Tris pH 7.5, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS (RIPA) and once in 140 mM NaCl, 20 mM Tris pH 7.5 (TBS). Where specified, these immunocomplexes were then incubated with further antiserum for 10 min at 4 °C before the kinase assay. Kinase assays were performed as follows. Immunocomplexes were resuspended in 20 μ l of 2.5 mM MnCl₂, 20 mM Tris pH 7.5 containing 5 μ Ci [γ -³²P]ATP per assay and incubated at 30 °C for 10 min. The reaction was stopped by the addition of 0.5 ml ice-cold TBS and immunocomplexes were collected by centrifugation, solubilized in 80 mM Tris pH 6.8, 2% SDS, 10% glycerol and electrophoresed on 9% polyacrylamide gels. In A, autoradiography was for 12 h with an intensifying screen; in B, autoradiography was for 8 h, and in C for 1 week. In C, samples to be analysed were cut from polyacrylamide gels which had not been fixed before drying and processed as described in ref. 20. Each gel slice was cut in half and the two halves placed in the slots of a 12% polyacrylamide gel. After incubation of the gel slices in 8 mM Tris pH 6.8, 0.2% SDS, 0.5% 2-mercaptoethanol, 10% glycerol for at least 15 min, *S. aureus* V8 protease was added and the gel electrophoresed at 15 mA for approximately 3 h.



rapidly on sucrose density gradients^{16,17}. Figure 2A confirms that when fractions from gradients of transformed cells were immunoprecipitated with hamster anti-T serum and kinase assays performed, middle T was labelled only in fractions sedimenting rapidly. To test whether the associated kinase activity of middle T as measured by the phosphorylation of exogenous substrate also sedimented rapidly, a similar gradient was analysed using a particular rat anti-T serum with which the immunoglobulin, but not middle T, becomes phosphorylated in the kinase reaction¹³. Figure 2B shows that the rat immunoglobulin was labelled predominantly in the same region of the gradient, demonstrating that the middle T associated kinase and the middle T acceptor activity both sedimented rapidly.

We also analysed density gradient fractionated cell extracts for pp60^{c-src} kinase activity. When normal cells were assayed with TBR serum, immunoglobulin phosphorylation was detected in fractions corresponding to a molecular weight (MW) of ~60,000 (Fig. 2D), suggesting that the active form of pp60^{c-src} kinase, like its viral counterpart, is monomeric¹⁸. By contrast, when fractions from the same gradient of polyoma virus-transformed cells shown in Fig. 2A were assayed, two overlapping peaks of activity were detected (Fig. 2C). One peak sedimented in approximately the same position as pp60^{c-src} from normal rat cells, whereas the second peak sedimented in approximately the same region as the middle T associated activity. Similar analyses of ³⁵S-methionine-labelled cell extracts showed that the sedimentation profile of pp60^{c-src} paralleled that of its associated kinase activity (data not shown). We conclude from these experiments that productive infection of mouse cells or transformation of rat cells by polyoma virus caused a fraction of pp60^{c-src} to sediment more rapidly than it did in uninfected cells. This heavier form had similar sedimentation properties to the fraction of middle T with associated protein kinase activity.

If middle T and pp60^{c-src} form a stable complex with one another, it should be possible to co-precipitate at least a fraction of either of the proteins by using an antibody directed against the other. Indeed, we showed in Fig. 1 that middle T was present in immunoprecipitates formed using TBR serum. We next sought direct evidence for co-precipitation of pp60^{c-src} and middle T by immunoprecipitation of metabolically labelled cell extracts. However, because the amounts of pp60^{c-src} and middle T are so low, the background of nonspecifically precipitated material was too high to allow definitive identification of the proteins using normal immunoprecipitation conditions. A more stringent procedure, involving elution of immunocomplexes from *Staphylococcus aureus* with buffer containing 4 M magnesium chloride and recovery of the immunoprecipitates on a second batch of bacteria, reduced background precipitation, but we would expect it to have disrupted noncovalent associations between proteins. If this was the case, any dissociated proteins should have been present in the supernatant following the second immunoabsorption. The experiment shown in Fig. 3 tests this prediction.

³⁵S-Methionine-labelled extracts of polyoma virus-transformed cells were immunoprecipitated with anti-T serum, and material which did not bind to the second batch of bacteria was subsequently immunoprecipitated with TBR serum. If normal rat serum was used in the first step, no middle T was detected in the first immunoprecipitate (Fig. 3, lane 1) and no proteins were specifically immunoprecipitated by TBR serum in the second step (lane 3). However, when anti-T serum was used in the first step, middle T was detected (lane 2) and magnesium treatment released a 60,000-MW protein which was specifically immunoprecipitated with TBR serum (lane 4) and co-migrated with authentic pp60^{c-src} (lane 6).

Co-immunoprecipitation was also demonstrated using ³²P-phosphate-labelled cells. Because the background of non-specifically precipitated proteins was less, the magnesium elution procedure was unnecessary. Immunoprecipitation of extracts of ³²P-labelled polyoma virus-infected mouse cells with anti-T serum showed that two proteins with MWs of ~60,000

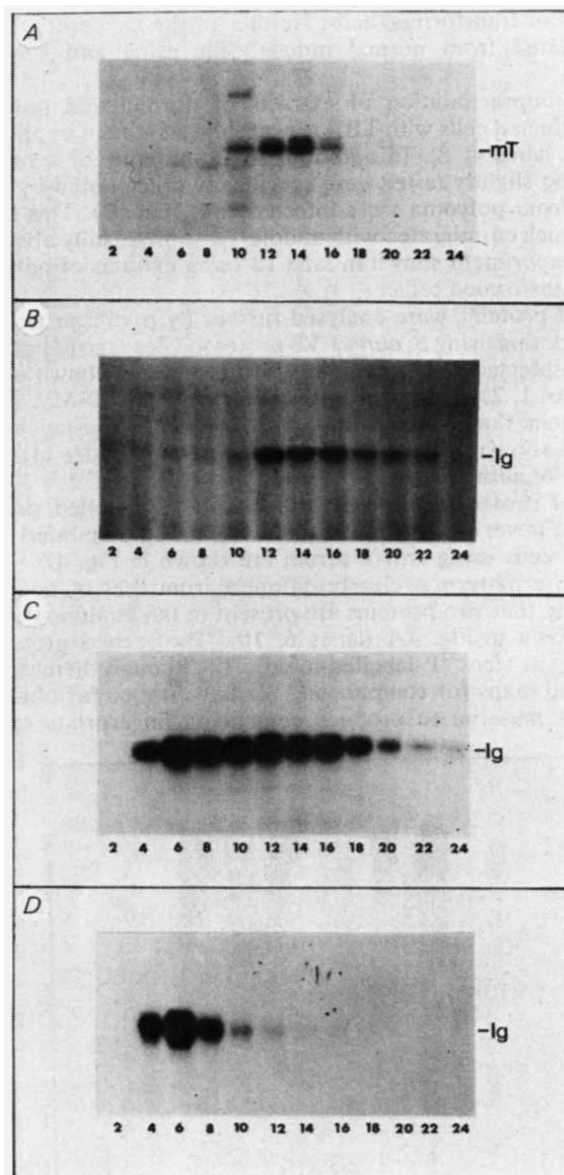


Fig 2 Sedimentation analyses of middle T and pp60^{c-src} associated kinase activities. A, 2.8-cell extract immunoprecipitated with hamster anti-T serum; B, 2.8-cell extract immunoprecipitated with a rat anti-T serum with which only the immunoglobulin becomes phosphorylated¹³; C, 2.8-cell extract immunoprecipitated with TBR serum; D, Rat 1 cell extract immunoprecipitated with TBR serum.

Methods: Extracts of Rat 1 and 2.8 cells were prepared as described in the legend to Fig. 1, and fractionated on 5-ml continuous gradients of 5–20% (w/v) sucrose in 150 mM NaCl, 1 mM dithiothreitol, 20 mM Tris pH 8, 0.1% NP40, 1% Trasylol. Centrifugation was in a Beckman SW55 rotor at 54,000 r.p.m. for 6 h at 4 °C. Twenty-four fractions were collected from each gradient and alternate fractions diluted with buffer C, immunoprecipitated and assayed for kinase activity as described in Fig. 1. Sedimentation was from left to right. The same gradient fractions were used for the analyses shown in A and C, but the analysis in B was from a separate experiment. The following molecular weight markers were analysed in a parallel gradient: ovalbumin, 43,000, fraction 4; bovine serum albumin, 67,000, fraction 7; aldolase, 160,000, fraction 11; influenza virus haemagglutinin, 225,000, fraction 14. In each case autoradiography was for 12 h with an intensifying screen.

were specifically precipitated (Fig. 4, lane 6) compared with control serum (lane 5). The presence of the two bands is perhaps more clearly seen in the analysis shown in lanes 9 and 10, using extracts of transformed cells. Neither of the two proteins was precipitated from normal mouse cells using anti-T serum (lane 2).

Immunoprecipitation of extracts of normal and polyoma virus-infected cells with TBR serum showed a band of pp60^{c-src} (Fig. 4, lanes 4, 8). In addition, small amounts of a protein migrating slightly faster were specifically precipitated by TBR serum from polyoma virus-infected cells (lane 8). This lower band which co-migrated with middle T is more readily observed in the experiment shown in lane 12 using extracts of polyoma virus-transformed cells.

These proteins were analysed further by partial proteolytic fingerprinting using *S. aureus* V8 protease. Cleavage of pp60^{c-src} from uninfected cells by two levels of protease is shown in Fig. 4B (lanes 1, 2). Proteolytic cleavage of the 60,000-MW phosphoprotein that co-precipitated with middle T using anti-T serum is shown in Fig. 4B (lanes 3, 4). The maps are identical to those of authentic pp60^{c-src}.

Partial proteolysis fingerprints of the ³²P-labelled protein from the lower band of the doublet immunoprecipitated from infected cells using anti-T serum are shown in Fig. 4B (lanes 5, 6). The pattern is clearly different from that of pp60^{c-src}, indicating that two proteins are present in the immunoprecipitates shown in Fig. 4A (lanes 6, 10). The second protein is probably *in vivo* ³²P-labelled middle T, although there are no published maps for comparison. We have not so far obtained sufficient material to produce convincing fingerprints of the

phosphoprotein that co-migrated with middle T in TBR serum precipitates of polyoma virus-infected cells. However, we have little doubt that this protein is middle T, because the experiment shown in Fig. 1B has already demonstrated the presence of middle T in such precipitates.

The data presented above strongly suggest that in lysates of cells productively infected with or transformed by polyoma virus, a fraction of middle T antigen is present in a complex with pp60^{c-src}. The complex can be isolated by immunoprecipitation using antibodies that react with either middle T or pp60^{c-src}. The proteins present in the complex can be dissociated with chaotropic agent to give the individual components which

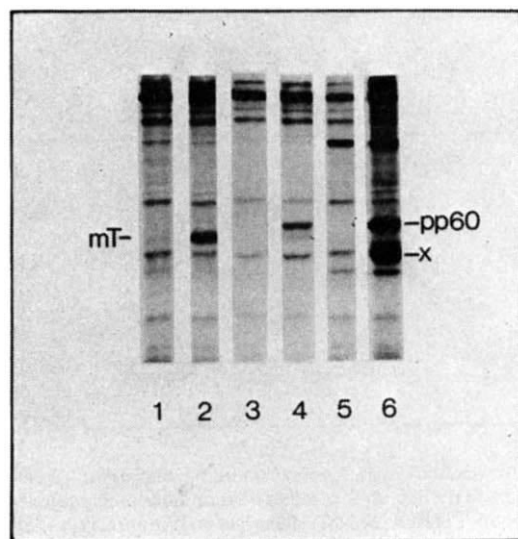


Fig. 3 Immunoprecipitation of ³⁵S-methionine-labelled pp60^{c-src} and middle T. Lane 1, normal rat serum; lane 2, rat anti-T serum; lane 3, material eluted from the sample in lane 1 immunoprecipitated with TBR serum; lane 4, material eluted from the sample in lane 2 immunoprecipitated with TBR serum; lane 5, normal rabbit serum; lane 6, TBR serum.

Methods: A 35-mm dish of 2.8 cells was labelled with 750 μ Ci ³⁵S-methionine and extracts prepared, immunoprecipitates formed and washed as described in the legend to Fig. 1, except that the lysis buffer used was 20 mM sodium phosphate, pH 7.2, 100 mM NaCl, 1 mM EDTA, 1% NP40, 1% Trasylol. Bound proteins were eluted with a solution containing 4 M MgCl₂ and immunocomplexes collected with a fresh batch of bacteria¹². Where indicated, the supernatants from these immunoprecipitates were then immunoprecipitated as follows. Antibody was added and the mixture incubated on ice for 30 min, then a fresh aliquot of *S. aureus* was added and incubation continued for a further 30 min. Immunocomplexes were collected by centrifugation and washed once in RIPA buffer and once in TBS before elution into sample buffer and gel electrophoresis. The band labelled X is probably a proteolytic degradation product of pp60^{c-src} similar to that described for pp60^{v-src} (ref. 21). Fluorography was for 1 week.

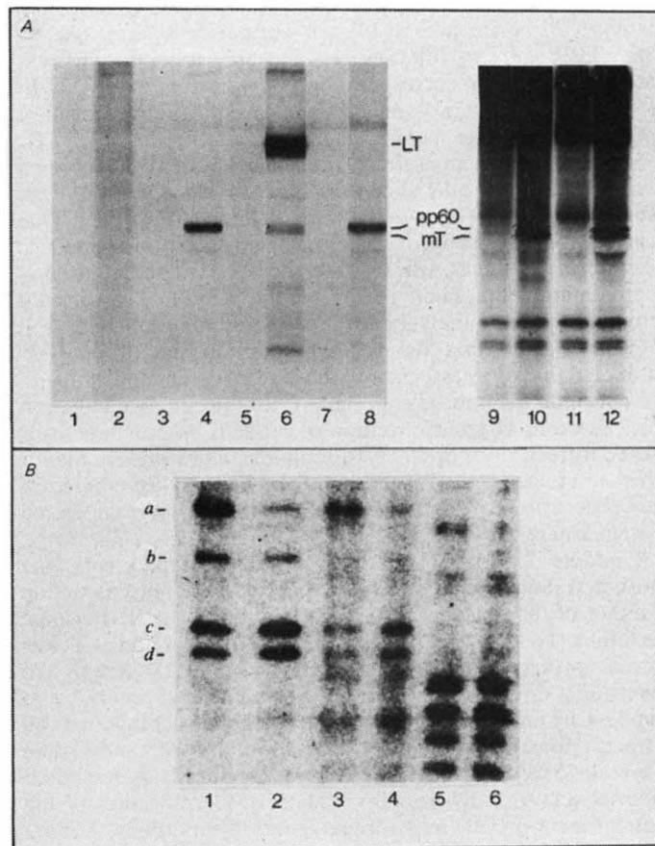


Fig. 4 Immunoprecipitation of ³²P-labelled pp60^{c-src} and middle T. **A**, Analysis of middle T and pp60^{c-src} labelled with ³²P *in vivo*. Lane 1, 3T6 cells, normal rat serum; lane 2, 3T6 cells, rat anti-T serum; lane 3, 3T6 cells, normal rabbit serum; lane 4, 3T6 cells, TBR serum; lane 5, polyoma virus-infected 3T6 cells, normal rat serum; lane 6, polyoma virus-infected 3T6 cells, rat anti-T serum; lane 7, polyoma virus-infected 3T6 cells, normal rabbit serum; lane 8, polyoma virus-infected 3T6 cells, TBR serum; lane 9, 2.8 cells, normal rat serum; lane 10, 2.8 cells, rat anti-T serum; lane 11, 2.8 cells, normal rabbit serum; lane 12, 2.8 cells, TBR serum. **B**, Analysis of phosphoproteins by one-dimensional peptide mapping. Lane 1, pp60^{c-src} from A, lane 4, 40 ng V8 protease; lane 2, pp60^{c-src} from A, lane 4, 200 ng V8 protease; lane 3, 60,000-MW protein from A, lane 6, 40 ng V8 protease; lane 4, 60,000-MW protein from A, lane 6, 200 ng V8 protease; lane 5, 55,000-MW protein from A, lane 6, 40 ng V8 protease; lane 6, 55,000-MW protein from A, lane 6, 200 ng V8 protease.

Methods: In A, 3T6 cells, polyoma virus-infected 3T6 cells, and 2.8 cells were incubated in phosphate-free medium for 2 h, then 1 mCi carrier-free ³²P was added to the cultures and incubation continued for a further 4 h. Extracts were made and immunocomplexes formed as described in Fig. 3 legend. The immunoprecipitates were washed 3 times in RIPA buffer and once in TBS before gel electrophoresis. Autoradiography was for 24 h with an intensifying screen. In B analysis was as described in the legend to Fig. 1, except that electrophoresis was on a 14% polyacrylamide gel. The four characteristic fragments of pp60^{c-src} are labelled a-d¹². Autoradiography was for 3 weeks with two intensifying screens.

are then precipitated separately using homologous antisera. The complex can also be detected on sucrose density gradients and seems quite stable, withstanding repeated washing with detergent containing buffers, 0.5 M NaCl and 10 mM EDTA. Furthermore, the complex can be detected in extracts of cells lysed in a variety of conditions including RIPA buffer and high concentrations of NP40 (unpublished observations).

An alternative explanation of our results is that the co-sedimentation of pp60^{c-src} with the kinase-active fraction of middle T is fortuitous, and that the antibodies we use cross-react. In other experiments to be described in detail elsewhere, we have used two other TBR sera and two other sera with activity against middle T, one of which was immunoaffinity purified antibody raised against a defined hexapeptide from the carboxy-terminus of middle T. We consider it extremely unlikely that all these sera cross-react. First, none of the antisera directed against middle T immunoprecipitate pp60^{c-src} from uninfected or untransformed cells, and second, middle T synthesized *in vitro* using polyoma virus-specific mRNA is not precipitated by TBR serum (unpublished observations).

Middle T has an associated tyrosine kinase activity *in vitro*. The data presented here suggest that this may be a property of that fraction of middle T associated with pp60^{c-src}. We propose, therefore, that middle T itself is not a protein kinase, but that the activity measured *in vitro* is, at least in part, a property of the associated pp60^{c-src}.

In agreement with previously published reports^{16,17} we find that the kinase active fraction of middle T sediments with an apparent MW of ~220,000. Further experiments will be needed to determine whether: (1) pp60^{c-src} and middle T are the only proteins present in the complex; (2) the complex exists *in vivo*, and (3) middle T is also able to associate with other cellular proteins. However, preliminary analysis of various middle T mutants has shown an excellent correlation between the transforming activity of the virus and the presence of the complex between pp60^{c-src} and middle T *in vitro*. pp60^{c-src} has been conserved during evolution and this argues that it serves an essential role in normal cells⁹. It is conceivable that by binding to pp60^{c-src} middle T is able to modulate either the activity or the specificity of the enzyme, and that this may play a part in transformation.

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1. Tooze, J. *DNA Tumour Viruses* 2nd edn, Pt 2 (Cold Spring Harbor Laboratory, New York, 1980).
2. Ely, B. K. & Smith, A. E. in *Advances in Viral Oncology* Vol. 3, 3–30 (Raven, New York).
3. Treisman, R., Novak, U., Favaloro, J. & Kamen, R. *Nature* **292**, 595–600 (1981).
4. Smith, A. E., Smith, R., Griffin, B. & Fried, M. *Cell* **18**, 915–924 (1979).
5. Eckhart, W., Hutchinson, M. A. & Hunter, T. *Cell* **18**, 925–933 (1979).
6. Schaffhausen, B. S. & Benjamin, T. L. *Cell* **18**, 935–946 (1979).
7. Ito, Y., Brocklehurst, J. R. & Dulbecco, R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4666–4670 (1977).
8. Sefton, B. M., Hunter, T., Beemon, K. & Eckhart, W. *Cell* **20**, 807–816 (1980).
9. Weiss, R., Teich, N., Varmus, H. E. & Coffin, J. *J. RNA Tumour Viruses* 2nd edn (Cold Spring Harbor Laboratory, New York, 1982).
10. Collett, M. S. & Erikson, R. L. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2021–2024 (1978).
11. Levinson, A. D., Oppermann, H., Levintow, L., Varmus, H. E. & Bishop, J. M. *Cell* **45**, 561–572 (1978).
12. Oppermann, H., Levinson, A. D., Varmus, H. E., Levintow, L. & Bishop, J. M. *Proc. natn. Acad. Sci. U.S.A.* **76**, 1804–1808 (1979).
13. Smith, A. E., Fried, M., Ito, Y., Spurr, N. & Smith, R. *Cold Spring Harb. Symp. quant. Biol.* **44**, 141–147 (1980).
14. Chinkers, M. & Cohen, S. *Nature* **290**, 516–519 (1981).
15. Kudlow, J. E., Buss, J. E. & Gill, G. N. *Nature* **290**, 519–521 (1981).
16. Schaffhausen, B. S. & Benjamin, T. L. in *Protein Phosphorylation* Vol. 2 (eds Rosen, O. M. & Krebs, E. G.) 1281–1298 (Cold Spring Harbor Laboratory, New York, 1981).
17. Walter, G., Hutchinson, M. A., Hunter, T. & Eckhart, W. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4025–4029 (1982).
18. Levinson, A. D., Oppermann, H., Varmus, H. E. & Bishop, J. M. *J. biol. Chem.* **255**, 11973–11980 (1980).
19. Brugge, J. S. & Erikson, R. L. *Nature* **269**, 346–348 (1977).
20. Oppermann, H., Levinson, A. D. & Varmus, H. E. *Virology* **108**, 47–70 (1981).
21. Courtneidge, S. A., Levinson, A. D. & Bishop, J. M. *Proc. natn. Acad. Sci. U.S.A.* **77**, 3783–3787 (1980).

Not more than 117 base pairs of 5'-flanking sequence are required for inducible expression of a human IFN- α gene

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Interferon genes are usually only expressed after induction¹. In the accompanying paper we have shown that the accumulation of mRNA after viral induction is due to activation of transcription, rather than to reduction of turnover, and that the regulation of the α -interferon (IFN- α) gene is mediated by a segment of 5'-flanking region of not more than 700 base pairs (bp)². To delineate the sequences required for induction, a set of 5' deletion mutants of the human IFN- α_1 gene was constructed and the expression of the truncated genes in mouse L cells was monitored after viral or mock infection. We report that not more than 117 bp of 5'-flanking sequence were required for induced expression of the gene. A purine-rich sequence of 42 bp located immediately downstream of position -117 is highly conserved in all known human α -interferon genes.

A 7.5-kilobase (kb) *Bam*HI fragment containing the human IFN- α_1 gene and 5.4 kb of 5'- and 1.2 kb of 3'-flanking sequences³ was ligated into the *Pvu*II site of pBR322. The resulting plasmid, pD6, in which the orientation of the interferon gene was opposite to that of the β -lactamase gene (Fig. 1A), was used to construct 5' deletion mutants, essentially as described by Frischauf *et al.*⁴. In short, the plasmid was partially digested with DNase I⁵ to yield about equal amounts of forms I, II and III DNA, and ligated to *Bam*HI linkers. Digestion with *Bam*HI linearized residual forms I and II DNA, while *Bam*HI-linked form III DNA was cleaved into two fragments. The mixture was further digested with *Sal*I to cleave full-length form III molecules and prevent their subsequent recircularization (see Fig. 1). The DNA preparation was fractionated on an agarose gel, and DNA fragments of the desired length (10–4.4 kb) were circularized with T4 DNA ligase and cloned. About 150 clones were picked and a set of mutants with deletions extending into the 5'-flanking region of the IFN- α_1 gene was identified by restriction analysis. The end points of deletions extending to position -166 or beyond (Fig. 1) were determined by sequencing. Each deletion mutant was cleaved at the unique *Hind*III site and concatenated with *Hind*III-cleaved pTKM-104. This plasmid consists of pBR322⁶ with a 2-kb *Pvu*II fragment comprising the herpes simplex virus (HSV) thymidine kinase (*tk*) gene⁷ inserted into its *Pvu*II site. The concatenates were introduced into TK⁻ mouse L cells^{8,9}, transformed TK⁺ cells were selected in hypoxanthine-aminopterin-thymidine (HAT) medium¹⁰ and about 1,000 colonies from each transformation experiment were pooled. Replicate dishes of pooled cells were grown to confluency and induced with Newcastle disease virus (NDV) or mock-induced 1.5–2 days later. Expression of the human interferon gene was measured by S₁ mapping^{11,12}, because it is important to score only for correctly initiated transcripts, and not those which might have originated by aberrant synthesis¹³. Human interferon activity after induction was very low¹⁴, partly because the specific activity of human IFN- α_1 is 20 times lower than that of other human α -interferons¹⁵. RNA was isolated 11 h after the beginning of induction or mock induction; the probe used for S₁ mapping was a 464-bp *Bam*HI-*Bgl*II fragment derived from a deletion mutant (end point -131), which was 5'-³²P-labelled at the *Bgl*II end of the minus strand and spanned the cap site.

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Figure 2 (lanes w-z and w'-z') shows that poly(A)⁺ RNA from interferon-producing human leukocytes protected a probe fragment of ~330 bp. No such signal was observed with RNA from induced or mock-induced mouse L cells transfected with the *tk*⁻ gene alone (lane o), showing that there was no cross-

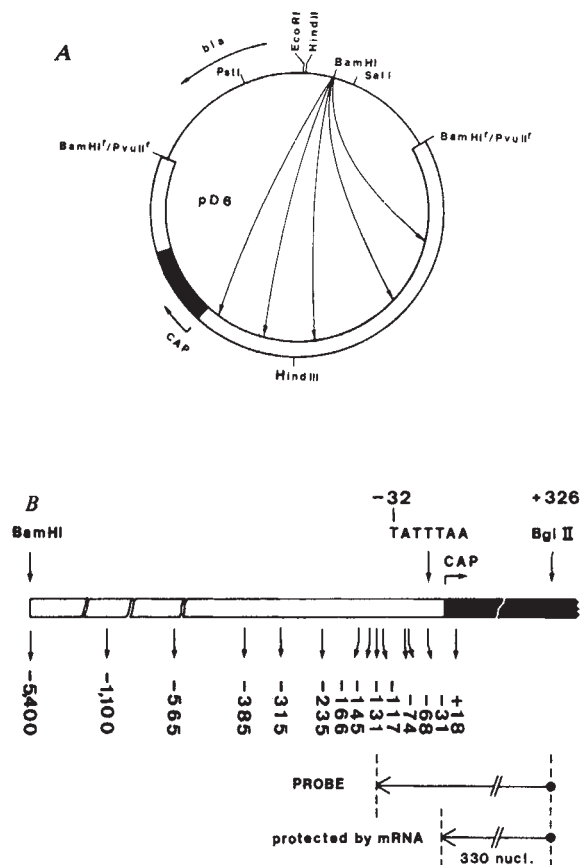


Fig. 1 Construction of 5' deletion mutants of the human IFN- α_1 gene. **A**, Plasmid pD6 contains a DNA segment comprising the human IFN- α_1 gene. Deletions removing segments of the 5'-flanking region were generated by inserting *Bam*HI linkers at random and deleting the DNA segment between the *Bam*HI site in the pBR322 moiety and the inserted linker⁴, as indicated by the arrows. **B**, Map of the IFN- α_1 gene with the end points of the deletions. The deletions extend from the *Bam*HI site in pBR322 (top arrow at left) to a *Bam*HI linker at the positions indicated by the bottom arrows. The numbers indicate the map position of the most upstream nucleotide of the residual 5'-flanking sequence relative to the cap site, which is assumed to be 70 nucleotides upstream of the initiator AUG³. The end points mapping at positions +18 to -166 were determined by nucleotide sequence analysis³⁹ from the linker *Bam*HI site. Open boxes, 5'- and 3'-flanking sequences of the human IFN- α_1 gene; solid box, IFN-coding region; thin line, pBR322 sequences. The long arrows extend from the *Bam*HI site in pBR322 to a few of the sites into which *Bam*HI linkers were inserted.

Methods: The 7.5-kb *Bam*HI fragment containing the human IFN- α_1 gene and 5.4 and 1.2 kb of 5'- and 3'-flanking sequences, respectively³, was bluntly with S₁ nuclease and Klenow DNA polymerase⁴⁰ and ligated into the *Pvu*II site of pBR322 to yield plasmid pD6. This plasmid was subjected to a limited digestion with DNase I in the presence of manganese ions^{4,5}. The ends were bluntly with Klenow DNA polymerase and kinased *Bam*HI linkers (5'-pCCGGATCCGG/3'-GGCCTAGGCC) attached⁴⁰. The DNA was cleaved with *Bam*HI and *Sal*I, and fractionated on a 0.7% low melting temperature (LMT) agarose gel. The DNA from individual gel slices (corresponding to fragment lengths of 10–4.4 kb) was circularized with T4 DNA ligase⁴, cloned in *Escherichia coli* HB101 and the resulting plasmids were characterized by restriction analysis with *Bam*HI and *Pvu*II. The deletion end points of a set of selected plasmids (see **B**) was determined by sequencing³⁹.

hybridization of the human probe with mouse interferon RNA. RNA from induced cells transformed with the modified human IFN- α_1 genes gave a strong signal indistinguishable from that of human leukocyte RNA, provided that the transcription unit was preceded by 117 bp or more of 5'-flanking sequence. The steady-state level of the correctly initiated transcripts after induction was estimated to be approximately 10–20 molecules per cell. These values were calculated taking into account the amount of RNA assayed, the RNA content of a cell (25 pg)¹⁶, the molecular weight of IFN- α_1 mRNA (330,000) and the radioactivities of the 330-nucleotide bands from the samples relative to those from a poly(A)⁺ RNA preparation from induced human leukocytes, which contained about 0.4% IFN- α_1 RNA (determined by N. Mantei). The values for the number of strands per cell represent average values, as they were obtained from pooled clones which probably expressed the human interferon gene to variable extents¹⁴. After mock induction no correct transcripts (<0.1 molecules per cell) were detected, except in the case of cells transformed by the gene with only 117 bp of 5'-flanking region. These not only had an exceptionally high transcript level after induction (~80 copies per cell), but also a low level (~0.1–0.3 molecules per cell) of correctly initiated transcripts after mock induction (Fig. 2j). Mutants with 74–31 bp of 5'-flanking sequences had a very low level of correctly initiated RNA molecules, whether they were induced or mock-induced.

Strong additional signals due to larger probe fragments appeared in all cases. These signals are probably mainly due to transcripts originating upstream of the cap site and to a lesser extent, if at all, to renatured probe. This was evident whenever the deletion end point of the probe DNA was located upstream of the deletion end point of the transforming DNA. In these cases (Fig. 2, lanes j–n) the transcripts originating upstream of the cap site did not protect the entire length of the probe and generated a signal distinct from that of the renatured probe (Fig. 2, lanes P).

Two further observations reinforce the significance of the response of the human IFN- α_1 gene in mouse cells to viral infection. First, treatment of the transformed cells with NDV caused induction of mouse interferon at similar levels in all transformed cell lines, including those where the 5' truncated human gene was not inducible (data not shown). Second, the level of 'upstream transcripts', in contrast to that of correctly initiated transcripts, was always lower in virus-infected than in mock-infected cells (Fig. 2, lanes a–n). The levels of other mRNAs were also reduced in virus-infected cells. S₁ mapping of HSV TK RNA (data not given) showed that all non-induced samples had about the same level of *tk* transcripts, and that this level was reduced about three- to fivefold 11 h after NDV infection. Similarly, NDV infection reduced the level of rabbit β -globin transcripts in mouse L cells transformed with the cognate gene². These results suggest that the levels of many if not all mRNAs are reduced after infection with NDV, perhaps due to general inhibition of transcription or accelerated degradation, and that in the case of interferon mRNA this reduction is offset by a strong stimulation of the interferon gene promoter. It has been shown previously that overall RNA synthesis in L cells is reduced by NDV infection^{17,18}.

We conclude from our experiments that infection of mouse L cells with NDV selectively stimulates correct transcription of the exogenous human IFN- α_1 gene and that 117 bp of 5'-flanking sequence are sufficient to mediate this induction. These findings are analogous to those made for the murine metallothionein I promoter¹⁹ and the *Drosophila* heat shock (*hsp*70) promoter²⁰, where 90 and 66 bp of 5'-flanking sequence, respectively, are sufficient for induction of transcription.

As removal of all but 117 bp of the IFN- α_1 5'-flanking sequence results in a low level of correctly initiated transcripts in non-induced cells, it is possible that the sequences normally present upstream of position -117 repress transcription or that the pBR322 sequences drawn downstream by the deletions promote a low level of transcription.

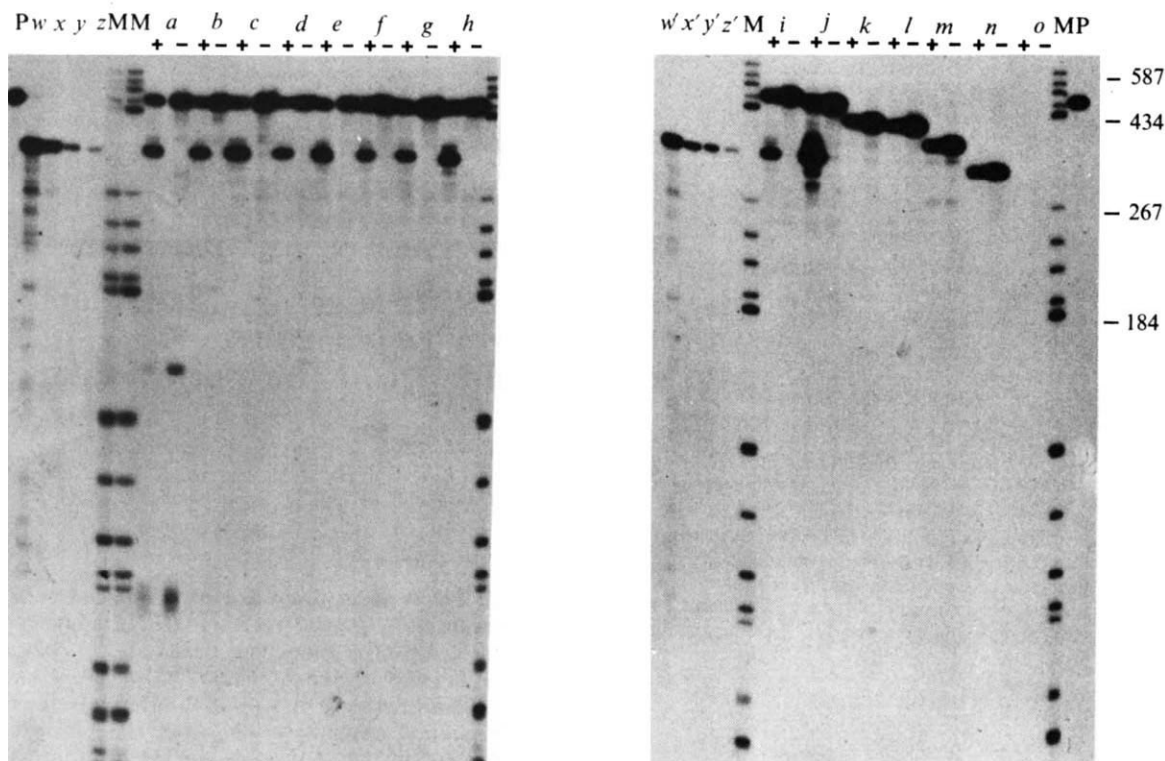


Fig. 2 S₁ analysis of human IFN- α_1 RNA from transformed mouse cells. Mouse L cells were transformed with each of the truncated IFN- α_1 genes shown in Fig. 1B, infected or mock-infected, and poly(A)⁺ RNA prepared after 11 h. S₁ analysis was carried out with the 5'-³²P-labelled minus-strand probe shown in Fig. 1B. Lanes w, x, y, z: 20, 6.6, 2 and 0.66 ng, respectively, of poly(A)⁺ RNA from interferon-producing human leukocytes; lanes w', x', y', z': 16, 4, 2 and 0.5 ng, respectively, of poly(A)⁺ RNA from interferon-producing human leukocytes; lanes a-n: poly(A)⁺ RNA from mouse cells transformed with the various 5' deletion mutants of the human IFN- α_1 gene, either induced (+) or mock-induced (-). Deletion end points: a, -5,400; b, -1,100; c, -565; d, -385; e, -315; f, -235; g, -166; h, -145; i, -131; j, -117; k, -74; l, -68; m, -31; n, +18. o, RNA from mouse cells transfected with linearized pTKM-104 only; lanes M, pBR322 digested with *Bsp*I and 5'-³²P-labelled; lanes P, undigested probe.

Methods: Concatenates were prepared by cleaving with *Hind*III the HSV *tk* gene-containing plasmid pTKM-104 (see text) and ligating it to each of the *Hind*III-cleaved 5' deletion mutants of the human IFN- α_1 gene listed in Fig. 1B, in a molar ratio of 1:6. Plasmid pD6 (which has two *Hind*III sites) was ligated via the *Bam*HI site (see Fig. 1A) to *Bam*HI-cleaved pTKM-104. Three 9-cm dishes of mouse LMTK⁻ cells were transfected with each DNA preparation (per dish: 0.1 pmol of pTKM-104, 0.6 pmol of the deletion mutant DNA and 18 μ g salmon sperm DNA added as carrier, by the calcium phosphate method)^{8,9}. Selection of *tk*⁺ clones in HAT medium¹⁰ was started after an additional 33 h of incubation, as described elsewhere¹⁴. Control cells were transfected with *Hind*III-cleaved plasmid pTKM-104. About 1,000 *tk*⁺ clones from each experiment were pooled and expanded to eight dishes. When the cells were about half confluent Dulbecco's minimal essential medium containing hypoxanthine (15 μ g ml⁻¹) and thymidine (5 μ g ml⁻¹) but no aminopterin was added (HT medium). Two days after confluency (3-4 days after addition of HT medium) the transformed cells were mock-infected or treated for 1 h with a dose of NDV established in preliminary experiments to give optimal induction of mouse IFN. RNA was extracted⁴¹ 11 h after virus addition and fractionated on oligo(dT)-cellulose⁴². For the preparation of the probe, plasmid IM 11-05, which contains an IFN- α_1 gene with a 5' deletion terminating at position -131 (see Fig. 1), was cleaved with *Bgl*II and the 5' termini were labelled with ³²P-phosphate⁴⁰. After *Bam*HI cleavage the 464-bp fragment was isolated on an LMT gel. Poly(A)⁺ RNA derived from 150 μ g of total RNA was hybridized to 10 nmol (40,000 c.p.m.) denatured probe as described elsewhere^{11,12}. After S₁ digestion the samples were analysed on a 5% denaturing polyacrylamide gel. Exposure was for 7 days at -70 °C with an Ilford intensifying screen. The bands were cut out and the radioactivity counted.

The finding (obtained in two independent transformation experiments) that the deletion mutant retaining only 117 bp of 5'-flanking sequence gave an approximately fivefold higher level of IFN- α_1 RNA after induction than deletion mutants with longer 5'-flanking regions (while the upstream transcripts were the same) argues that sequences upstream of position -117 might exert or mediate an inhibitory effect on transcription which is only partly relieved by induction. The low level of transcripts after induction in the case of mutants with deletions to position -74 and downstream from it could be due to the loss of essential components of the promoter, which in many genes extend to position -100 and beyond²¹⁻²⁵.

The sequence of the human IFN- α_1 gene between position -117 and -40 (Fig. 3) is very purine-rich (75%); the sequence GAAAGTGG, positions -78 to -71, is repeated at position -56 to -49 and part of this sequence, GAAA, is repeated at positions -97 and -49. The 5'-flanking sequences of nine

distinct IFN- α genes²⁶ have a homologous stretch of 42 nucleotides (positions -116 to -75 in IFN- α) in which 62% of the positions are identical among the different interferon species (refs 3, 27-29, and K. Henco *et al.*, unpublished results); the neighbouring regions show far fewer conserved positions (Fig. 3). The human IFN- β gene, which resembles the IFN- α genes with regard to its inducibility by virus³⁰⁻³³, also has a purine-rich region at positions -105 to -63 (relative to the putative cap site)³⁴⁻³⁷ which shows a clear homology to that of the IFN- α genes. Tavernier *et al.*³⁸ have carried out deletion mapping of the human IFN- β gene, using an SV40-linked transient expression system in monkey cells and determining human IFN- β production after poly(IC) induction. They concluded that deletions extending to a position between -144 and -186 precluded expression of the IFN- β gene. On the other hand, T. Taniguchi and his colleagues (personal communication), using a methodology similar to ours, found that the critical

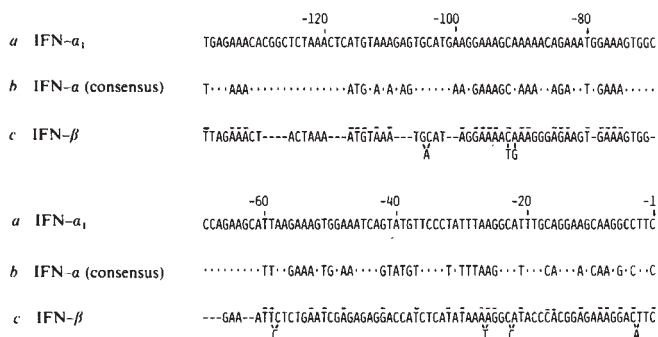


Fig. 3 Comparison of nucleotide sequences of 5'-flanking regions of human interferon genes. *a*, IFN- α_1 sequence between positions -1 to -138 (ref. 3). The numbering is relative to the putative cap site (+1). Nagata *et al.*³ numbered the putative cap site as -69, relative to the AUG initiator triplet. *b*, Nucleotide positions conserved among nine distinct IFN- α genes: α_1 (ref. 3), α_4 (K. Henco and A. Schamböck, unpublished), α_5 (J. Fujisawa, unpublished), α_6 (J. Schmid, unpublished), α_7 (T. Kovacic, unpublished), α_{10} (J. Brosius, unpublished), $\lambda 2h$ (ref. 27), $\lambda 2cl$ (ref. 27) and $\lambda \alpha 2$ (ref. 28). Only nucleotides identical in all sequences are indicated; a dot indicates absence of a common residue. *c*, The IFN- β sequence is from Ohno *et al.*³⁴ and Degrove *et al.*³⁵. Dashes indicate 'gaps' introduced to align the IFN- α consensus sequence. Positions identical with the IFN- α consensus sequence are overlined.

cutoff region lay between positions -125 and -117.

In any event it should be borne in mind that this type of deletion mapping by itself merely locates DNA regions essential for transcription in general, and gives no information on specific sequences required for induction. However, in conjunction with the experiments reported by Weidle and Weissmann², we conclude that inducible control of the IFN- α gene is due to sequences located between positions -117 and -6.

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- Stewart, W. E. II *The Interferon System* (Springer, New York, 1979).
- Weidle, U. & Weissmann, C. *Nature* **303**, 442-446 (1983).
- Nagata, S., Mantei, N. & Weissmann, C. *Nature* **287**, 401-408 (1980).
- Frischaut, A. M., Garoff, H. & Lehrach, H. *Nucleic Acids Res.* **8**, 5541-5549 (1980).
- Heffron, F., So, M. & McCarthy, B. J. *Proc. natn. Acad. Sci. U.S.A.* **75**, 6012-6016 (1978).
- Sutcliffe, J. G. *Cold Spring Harb. Symp. quant. Biol.* **43**, 77-90 (1979).
- Wilkie, N. M. *et al. Nucleic Acids Res.* **7**, 859-877 (1979).
- Graham, F. L. & van der Eb, A. J. *Virology* **52**, 456-467 (1973).
- Wigler, M., Pellicer, A., Silverstein, S. & Axel, R. *Cell* **14**, 725-731 (1978).
- Szybalski, W., Szybalska, E. H. & Ragni, G. *Monogr. natn. Cancer Inst.* **7**, 75 (1962).
- Berk, A. H. & Sharp, P. A. *Cell* **12**, 721-732 (1977).
- Weaver, R. F. & Weissmann, C. *Nucleic Acids Res.* **7**, 1175-1193 (1979).
- Dierks, P., van Ooyen, A., Mantei, N. & Weissmann, C. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1411-1415 (1981).
- Mantei, N. & Weissmann, C. *Nature* **297**, 128-132 (1982).
- Streuli, M. *et al. Proc. natn. Acad. Sci. U.S.A.* **78**, 2848-2852 (1981).
- Parsons, J. T., Coffin, J. M., Haroz, R. K., Bromley, P. A. & Weissmann, C. *J. Virol.* **11**, 761-774 (1973).
- Wilson, D. E. *J. Virol.* **2**, 1-6 (1968).
- Thacore, H. & Youngner, J. S. *J. Virol.* **6**, 42-48 (1970).
- Brinster, R. L., Chen, H. Y., Warren, R., Sarthy, A. & Palmiter, R. D. *Nature* **296**, 39-42 (1982).
- Pelham, H. R. B. *Cell* **30**, 517-528 (1982).
- Dierks, P. *et al. Cell* **32**, 695-706 (1983).
- McKnight, S. L. & Kingsbury, R. *Science* **217**, 316-324 (1982).
- Fromm, M. & Berg, P. *J. molec. appl. Genet.* **1**, 457-481 (1982).
- Hen, R., Sassone-Corsi, P., Corden, J., Gaub, M. B. & Chambon, P. *Proc. natn. Acad. Sci. U.S.A.* **79**, 7132-7136 (1982).
- Benoist, C. & Chambon, P. *Nature* **290**, 304-310 (1981).
- Weissmann, C. *et al. Phil. Trans. R. Soc. B299*, 7-28 (1982).
- Lawn, R. M. *et al. Science* **212**, 1159-1162 (1981).
- Lawn, R. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **78**, 5435-5439 (1981).
- Ulrich, A., Gray, A., Goeddel, D. V. & Dull, T. J. *J. molec. Biol.* **156**, 467-486 (1982).
- Ohno, T. & Taniguchi, T. *Nucleic Acids Res.* **10**, 967-977 (1982).
- Hauser, H. *et al. Nature* **297**, 650-654 (1982).
- Zinn, K., Mellon, P., Ptashne, M. & Maniatis, T. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4897-4901 (1982).
- Canaani, D. & Berg, P. *Proc. natn. Acad. Sci. U.S.A.* **79**, 5166-5170 (1982).
- Ohno, S. & Taniguchi, T. *Proc. natn. Acad. Sci. U.S.A.* **78**, 5305-5309 (1981).

- Degrave, W., Derynck, R., Tavernier, J., Haegeman, G. & Fiers, W. *Gene* **14**, 137-143 (1981).
- Houghton, H. *et al. Nucleic Acids Res.* **9**, 247-266 (1980).
- Lawn, R. M. *et al. Nucleic Acids Res.* **9**, 1045-1052 (1981).
- Tavernier, J., Gheysin, D., Duerinck, F., Van der Heiden, J. & Fiers, W. *Nature* **301**, 634-636 (1983).
- Maxam, A. & Gilbert, W. *Meth. Enzym.* **65**, 499-560 (1980).
- Maniatis, T., Fritsch, E. F. & Sambrook, J. (eds) *Molecular Cloning* (Cold Spring Harbor Laboratory, New York, 1982).
- Auffray, C. & Rougeon, F. *Eur. J. Biochem.* **107**, 303-314 (1980).
- Aviv, H. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1408-1412 (1972).

The 5'-flanking region of a human IFN- α gene mediates viral induction of transcription

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Usually only cells exposed to virus, double-stranded RNA or other inducers synthesize interferon (IFN)^{1,2}. Interferon mRNA appears 1-2 h after induction, peaks at 1.5-20 h and decays with a half life of about 30 min³⁻⁶. So far, it has not been determined whether induction of interferon is due to transient stabilization of a rapidly turning-over mRNA or to activation of transcription. To clarify this issue we transformed mouse L cells with a hybrid gene in which the 5'-flanking region of the human IFN- α_1 gene was followed by the rabbit β -globin transcription unit. Correctly initiated β -globin RNA appeared only after viral induction, with the kinetics described for interferon mRNA⁷. Cells transformed with the converse construction, or with the complete rabbit β -globin gene, constitutively produced correctly initiated transcripts; viral infection decreased the level of transcripts. We conclude that induction acts by activating transcription rather than by reducing turnover, and that the regulatory elements are contained in the 5'-flanking region of the interferon gene.

p Δ 25B contains the rabbit β -globin transcription unit preceded by 425 base pairs (bp) of 5'-flanking DNA⁸. An *Xba*I linker was inserted at position -9/-10 (numbering relative to the cap site), to yield p Δ 25B(*Xba*-10). pChr35-675 contains the human IFN- α_1 transcription unit preceded by about 675 bp of 5'-flanking region⁷. *Xba*I linkers were introduced at positions -5/-6 and -69/-70 (the numbering is relative to the cap site, which is 69 $\pm$ 3 nucleotides upstream from the AUG initiator triplet; here we designate as +1 the position given as -69 in ref. 7). The β -globin 5'-flanking segment between position -425 and the *Xba*I linker contains all elements required for expression in heterologous cells⁹. It was joined to the IFN- α_1 transcription unit at the *Xba*I site in position -5/-6, to yield pGI, positioning the IFN- α cap site 34 bp downstream of the first A residue of the β -globin gene ATA box.

In the converse construction, pIG-I, a fragment of about 675 bp of IFN- α_1 5'-flanking region, extending up to the *Xba*I linker at position -5/-6, was joined to the β -globin gene at the *Xba*I linker at -9/-10, positioning the cap site of the β -globin gene 43 bp downstream of the IFN- α_1 ATA box. In pIG-II, a fragment of IFN- α_1 5'-flanking sequence extending to the *Xba*I linker at position -69/-70 was linked to the β -globin DNA fragment; in this case, the hybrid gene contained no ATA box.

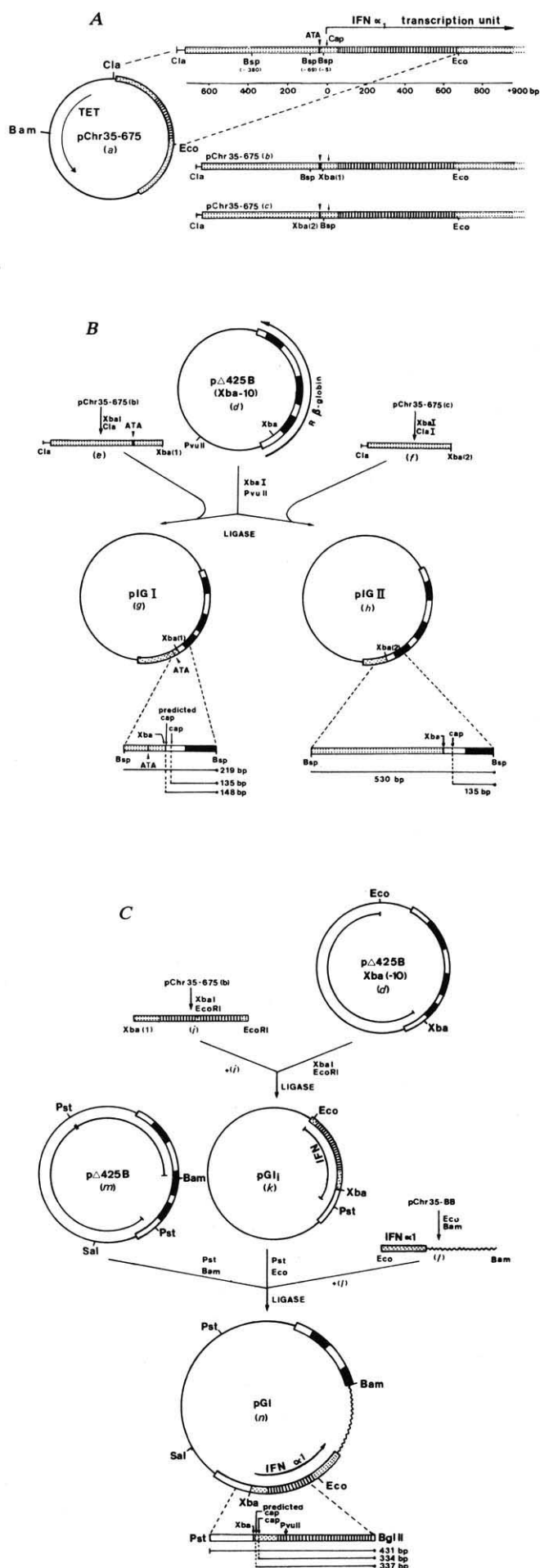
Mantei and Weissmann⁷ transformed thymidine kinase (TK) negative mouse L cells with concatenates containing the herpes simplex virus (HSV) *tk* gene and the human IFN- α_1 gene. After infection with Newcastle disease virus (NDV), but not after mock infection, correctly initiated IFN- α_1 transcripts appeared

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Mouse L cells were transformed with hybrid genes in which the β -globin transcription unit was joined either to an IFN- α_1 5'-flanking sequence comprising the ATA box (pIG-I) or lacking it (pIG-II). Transcripts of uninduced and induced cells were mapped with a 5'- 32 P-labelled cognate minus-strand probe spanning the putative cap site (Fig. 1). The 5' end of a β -globin transcript originating 30 bp downstream⁹ of the ATA box in pIG-I should map 13 bp upstream of the β -globin cap site (Fig. 2) and give an S₁ fragment of 148 bp (Fig. 1B), while authentic β -globin mRNA should give a 135-bp fragment. S₁ mapping (Fig. 3) with the probe derived from pIG-I gave the expected 135-bp signal for authentic rabbit β -globin mRNA, as well as

Fig. 1 Construction of hybrid rabbit β -globin-human IFN- α_1 genes. *A*, *Xba*I linkers were inserted into the 5'-flanking region of the IFN- α_1 gene (*a*) either downstream (*b*, *Xba*(1)) or upstream (*c*, *Xba*(2)) of the ATA box. *B*, The 5'-flanking region of the IFN- α_1 gene, either with (*e*) or without (*f*) the ATA box, was joined to the transcription unit of the β -globin gene (*d*) at the *Xba*I site, to yield pIG-I (*g*) and pIG-II (*h*), respectively. The *Xba*I site in the β -globin gene had been generated by inserting an *Xba*I linker into the *Pvu*II site at position -9/-10. *C*, The 5'-flanking region of the rabbit β -globin gene (*d*) was joined in a multi-step procedure to the transcription unit of the IFN- α_1 gene at the downstream *Xba*I site (*i*) to yield pGI (*n*). Stippled boxes, 5'-flanking, 5'- and 3'-noncoding regions of IFN- α_1 gene; wavy line, 3'-flanking region of the IFN- α_1 gene; hatched boxes, IFN- α_1 coding region; open boxes, introns, flanking and noncoding regions of the rabbit β -globin gene; smooth black lines, pBR322 sequences; arrowhead, ATA box. '*Xba*(1)' and '*Xba*(2)' refer to *Xba*I linkers inserted into the *Bsp*II site downstream and upstream of the ATA box, respectively. TET, tetracycline resistance gene. The horizontal lines at the bottom of *B* and *C* indicate the probes used for *S*₁ mapping and the fragments protected by correctly initiated transcripts.

Methods: A. Insertion of *Xba*I linkers into the *Bsp*II sites of pChr35-675. pChr35-675 (*a*) is a derivative of Z-pBR322 (*Pst*I)/Hifchr35-HB α (ref. 27) in which the fragment between the *Eco*RI site at -695 of the IFN 5'-flanking region and the *Eco*RI site of pBR322 was deleted and the resulting *Eco*RI site destroyed. After partial cleavage with *Bsp*II, linear DNA was isolated and ligated to *Xba*I linkers. After cleavage with *Xba*I, religation and transfection into *Escherichia coli* HB101 80 colonies were pooled and mixed plasmid DNA was prepared. Plasmids pChr34-675 (*b*) and (*c*) (*A*) were in this collection but were not isolated. **B.** Preparation of pIG-I and pIG-II. The mixed plasmid DNA was cleaved with *Cl*aI and the ends blunted with T4 DNA polymerase²⁸. After cleavage with *Xba*I, the 720-bp and 660-bp fragments comprising the 5'-flanking region with (*e*) and without (*f*) the ATA box, respectively, were isolated. p425B (*Xba*-10) (see *B*, *d*) which contained an *Xba*I linker in the *Pvu*II site at -9/-10 of the rabbit β -globin gene²⁹ was cleaved with *Xba*I and *Pvu*II, ligated to the 660- and 720-bp *Xba*I-*Cl*aI fragments, respectively, and cloned. Colonies containing the interferon sequence were identified by hybridization³⁰. The junction sequence of pIG-I (*g*) was confirmed by sequencing³¹. **C.** Preparation of pGI. The plasmid collection described in *A* was cleaved with *Xba*I and *Eco*RI and the 713-bp fragment (*i*) isolated and joined to the 5'-flanking region of the β -globin gene by ligation to the large *Xba*I-*Eco*RI fragment of p425B(*Xba*-10). The resulting plasmid pGI; (*k*) lacked part of the 3' noncoding region of IFN- α_1 . The IFN- α_1 transcription unit was reconstituted by joining the 3.5-kb *Pst*I and 580-bp *Pst*I-*Bam*HI fragments of p425B (*m*), the 804-bp *Pst*I-*Eco*RI fragments from pGI; (*k*) and the 1.5-kb *Eco*RI-*Bam*HI fragment (*l*) from pChr35-BB (which contained a 7.5-kb *Bam*HI fragment from the IFN- α_1 chromosomal DNA chr35 (ref. 27), in the *Bam*HI site of pBR322). The structure around the *Xba*I junction was confirmed by sequencing³¹.



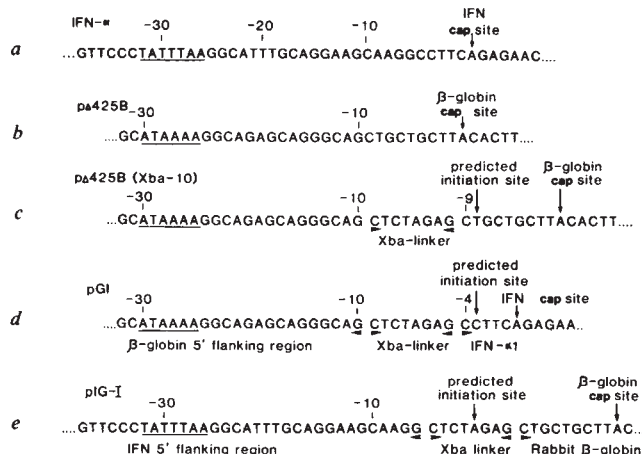


Fig. 2 Nucleotide sequences at the junction of the 5'-flanking region and the transcription unit of unmodified and hybrid genes. *a*, Human IFN- α_1 ; *b*, rabbit β -globin (p Δ 425B)⁸; *c*, rabbit β -globin DNA p Δ 425B(Xba-10); *d*, β -globin-IFN- α_1 hybrid (pGI); *e*, IFN- α_1 - β -globin hybrid (pIG-I). The ATA box is underlined. The numbering is relative to the cap site of the original sequences (see A and B).

a signal due to the renatured 219-bp probe (lanes *a*, *b*). Poly(A)⁺ RNA from the virus-treated (lane *d*) but not the mock-infected cells transformed with pIG-I (lane *c*) gave a strong signal at about 150 bp, as well as a 219-bp band due to renatured probe and possibly to transcripts originating upstream of position -69. About 10–25 correctly initiated transcripts per cell were present 11 h after induction, as estimated by comparing the intensity of the 148-bp band with that of the 135-bp band given by known amounts of authentic β -globin mRNA, and performing the calculation described earlier⁷. All samples from β -globin DNA-containing cells gave a strong 87–97-bp band due to β -globin transcripts protecting the probe up to position 40. Such transcripts were previously found in cells transformed by cloned rabbit β -globin genes and are attributed to abnormal initiation or to aberrant splicing^{9,12,13}. As the level of these abnormal transcripts was about the same for the induced and non-induced samples, the absence of the 148-nucleotide signal in the non-induced sample is not due to degradation or losses during RNA isolation or S₁ analysis.

Poly(A)⁺ RNA from induced and non-induced cells transformed with pIG-II gave no signals other than the 87–97-bp band due to abnormal transcripts, and a weak band in the position of completely protected probe. To ensure that induc-

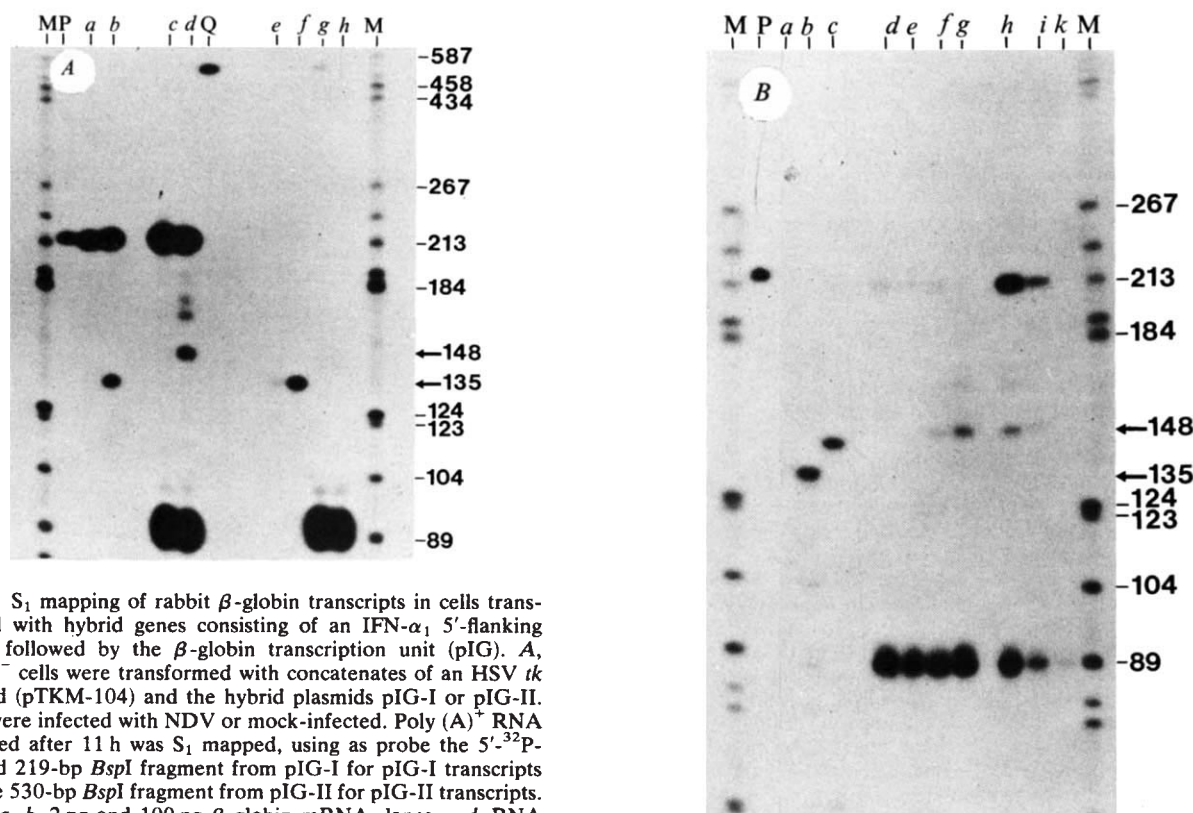


Fig. 3 S₁ mapping of rabbit β -globin transcripts in cells transformed with hybrid genes consisting of an IFN- α_1 5'-flanking region followed by the β -globin transcription unit (pIG). *A*, LMTK⁻ cells were transformed with concatenates of an HSV *tk* plasmid (pTKM-104) and the hybrid plasmids pIG-I or pIG-II. Cells were infected with NDV or mock-infected. Poly (A)⁺ RNA extracted after 11 h was S₁ mapped, using as probe the 5'-³²P-labelled 219-bp *Bsp*I fragment from pIG-I for pIG-I transcripts and the 530-bp *Bsp*I fragment from pIG-II for pIG-II transcripts. Lanes *a*, *b*, 2 pg and 100 pg β -globin mRNA; lanes *c*, *d*, RNA from cells transformed with pIG-I (*c*, mock-infected; *d*, NDV-infected); lanes *e*, *f*, 20 pg and 100 pg β -globin mRNA; lanes *g*, *h*, RNA from cells transformed with pIG-II (*g*, NDV-infected; *h*, mock-infected); lanes *P*, *Q*, undigested probes; lane *M*, pBR322 digested with *Bsp*I and 5'-³²P-labelled. *B*, LMTK⁻ cells transformed with concatenates of pIG-I and HSV *tk* DNA were induced with NDV, poly(A) RNA was prepared after different times and S₁ mapped. Lanes *a*, *b*, 20 pg and 200 pg β -globin mRNA, lanes *d*, *e*, *f*, *g*, *h*, *i*, *k*, RNA from cells extracted 0, 4, 8, 11, 14, 21 and 35 h, respectively, after infection with NDV. Lane *P*, undigested probe. Lane *c*, probe digested with *Xba*I; lane *M*, as above.

Methods: pTKM-104 contains the 2.05-kb *Pvu*II fragment from M2³² in the *Pvu*II site of pBR322. Two μ g pTKM-104 and 10 μ g of pIG-I or pIG-II were cleaved with *Cl*aI and concatenated with T4 DNA ligase¹². Two 9-cm dishes of mouse LMTK⁻ cells were transfected and *tk*⁺ clones selected^{7,12}. About 500 colonies were trypsinized, grown to confluency on one dish, split 1:4, and grown to confluency. One dish each was infected with the amount of NDV required for optimal induction of mouse interferon, or mock-infected. After 1 h the medium was changed, RNA was extracted⁸ 11 h later and poly(A)⁺ RNA prepared³³. To prepare the probes for S₁ mapping, pIG-I was digested with *Bsp*I, and pIG-II with *Bsp*I and *Fnu*DI, the 5' termini were ³²P-labelled³¹ and the desired *Bsp*I fragments isolated. The 219-bp fragment from pIG-I extends from +135 in the β -globin gene to -69 in the IFN- α_1 gene, the 530-bp *Bsp*I fragment pIG-II from +135 in the β -globin gene to about -380 in the 5'-flanking region of IFN- α_1 . The labelled minus-strand was isolated on a strand separation gel³¹. For further purification, about 2 pmol were annealed in 50 μ l 3 $\times$ SSC in the presence of 100 μ g ml⁻¹ denatured calf thymus DNA at 60°C for 24 h. Single-stranded DNA was recovered by hydroxylapatite chromatography at 60°C³⁴ and purified on DEAE-cellulose. For S₁ mapping, probe (0.03 pmol, 35,000 c.p.m.) was hybridized to poly(A)⁺ RNA in 80% formamide, digested with S₁ nuclease and analysed on a 5% polyacrylamide gel as described previously^{10,11}.

tion had been effective, mouse interferon mRNA was S_1 -mapped with a 5'-labelled murine IFN- α_2 probe (MuIFN- α_2 ; ref. 14)—no signal was found in the non-induced samples, while both induced samples gave strong signals (data not shown). These experiments showed that the IFN- α_1 5'-flanking region containing the ATA box, after induction with virus, directed the expression of β -globin RNA with the expected 5' terminus.

The level of induced transcripts in cells transformed with pIG-I was determined at different times after infection. No 148-bp fragment was detectable at time 0 or at 4 h after infection (Fig. 3B, lanes *d*, *e*); the signal was visible at 8 h, maximal at 11 h (lanes *f*, *g*) and decreased progressively at 14, 21 and 35 h (lanes *h*, *i*, *k*). The 87–97-bp signals due to abnormal β -globin transcripts remained constant until 14 h after infection, and then decreased, perhaps as a consequence of damage due to viral infection^{15,16}. An even more striking effect was observed for HSV *tk* transcripts which 11 h after infection were already diminished 10-fold (data not shown).

The fact that correctly initiated β -globin RNA appeared in cells transformed with pIG after viral infection could be due, at least in part, not to increased transcription, but to reduced turnover. To assess this possibility we examined the effect of virus infection on the expression of the rabbit β -globin and the human IFN- α_1 transcription units, both under the control of the rabbit β -globin promoter. Cells were transformed with concatenates containing either the normal rabbit β -globin gene (p4425B)⁸, the rabbit β -globin gene with an *Xba*I linker at position -9/-10 (p4425 (*Xba*-10)), or the IFN- α_1 transcription unit linked to the β -globin promoter (pGI), and mixtures of transformed cell lines were prepared as above.

S_1 analysis of poly(A) RNA from cells transformed with p4425B or p4425B (*Xba*-10) was with a 5'-³²P-labelled 210-bp *Bsp*I fragment (-75 to -135) from p4425B. Correctly initiated transcripts should protect a 135-bp fragment in the case of p4425B. In the case of p4425B (*Xba*-10), a fragment of ~143 bp was expected because initiation should be displaced about 8 bp upstream due to the *Xba*I linker between the ATA box and the cap site⁹ (see Fig. 2). Figure 4 (lanes *d*-*g*) shows the expected signals; the transcript levels, estimated as described above, were about 250 and 170 per cell in mock-infected cells transformed with p4425B and p4425B (*Xba*-10) respectively, and about half that in virus-infected cells. The transcripts derived from pGI were S_1 -mapped with a 5'-³²P-labelled minus-strand probe which extended from position +330 (IFN- α_1) to -99 (β -globin). RNA molecules initiating 30 bp downstream from the ATA box (that is, 4 bp upstream from the IFN- α_1 cap site (see Fig. 2)) should protect a 334-bp probe fragment. As shown in Fig. 4 (lanes *k*, *l*), the expected signals appeared in all cases; mock-infected cells contained about 1–2 and NDV-infected cells about 1 transcript per cell. In all cases, mouse IFN- α_1 mRNA was present at similar levels after virus infection and was absent after mock infection (data not given), showing that induction had been effective. We conclude that neither the IFN- α_1 nor the β -globin RNA was stabilized by virus infection.

Our experiments provide strong evidence that the induction of IFN- α_1 mRNA is primarily due to activation of transcription, that transcriptional control is exerted via the 5'-flanking region of the IFN- α_1 gene and that the rate of IFN- α_1 mRNA turnover is not reduced (and possibly even increased) after viral induction. We argue as follows. The level of β -globin mRNA in cells transformed with a β -globin gene decreased 11 h after induction; therefore, β -globin mRNA was not stabilized by induction. In drawing this conclusion we disregard the less likely possibility that induction stabilized β -globin transcripts but that this effect was more than offset by a reduction in β -globin transcription. The level of β -globin RNA in cells transformed with a hybrid gene consisting of the IFN- α_1 promoter and the β -globin transcription unit was zero before, and 10–25 transcripts per cell after induction. If this rise is not attributed to stabilization of β -globin mRNA, it must be due to activation of transcription. Cells transformed with a hybrid gene consisting

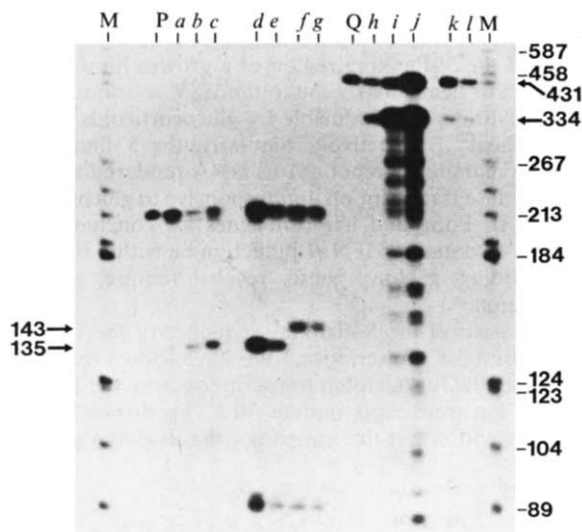


Fig. 4 S_1 mapping of RNA transcribed from a hybrid gene consisting of the rabbit β -globin 5'-flanking region followed by the IFN- α_1 transcription unit, or from the β -globin gene. LMTK⁻ cells were transformed with concatenates containing the HSV *tk* gene (pTKM-104) and the hybrid β -globin IFN- α_1 gene pGI (Fig. 1C), the rabbit β -globin gene p4425B⁸ or the β -globin gene with an *Xba*I linker at position -9/-10 (p4425B (*Xba*-10)); transformed cells were induced with NDV or mock-induced, and poly(A)⁺ RNA was prepared after 11 h. β -Globin transcripts (lanes *a*-*g*) were mapped with a 210-bp *Bsp*I fragment from p4425 spanning the cap region⁸, and human IFN- α_1 RNA (lanes *h*-*l*) with a 431-bp *Bgl*II-*Pst*I fragment spanning the cap region (see Fig. 1c). Lanes *a*, *b*, *c*, 40 pg, 120 pg and 200 pg rabbit β -globin mRNA, respectively; lanes *d*, *e*, poly(A)⁺ RNA from 100 μ g of total RNA from cells transformed with p4425B (*d*, mock-infected; *e*, NDV-infected); *f*, *g*, poly(A)⁺ RNA from cells transformed with p4425 (*Xba*-10) (*f*, mock-infected; *g*, NDV-infected); lanes *h*, *i*, *j*, 10 ng, 100 ng, and 300 ng, respectively, poly(A)⁺ RNA from IFN-producing leukocytes (containing ~0.4% IFN- α_1 mRNA); lanes *k*, *l*, poly(A)⁺ RNA from 400 μ g of total RNA from cells transformed with pGI (*k*, mock-infected; *l*, NDV-infected). Lane M, pBR322 digested with *Bsp*I and 5'-³²P-labelled.

Methods: Concatenation, transformation, infection and mock infection, and preparation of poly(A)⁺ RNA were as described in Fig. 3 legend. To prepare the probe for S_1 mapping of β -globin transcripts, plasmid p4425B was digested with *Bsp*I and *Fnu*DI and the 210-bp *Bsp*I fragment (+135 to -75) isolated. After treatment with Klenow DNA polymerase I and dTTP (ref. 28) to create 5' overhanging ends, the 5' termini were ³²P-labelled³¹. To generate the probe for mapping interferon transcripts, pGI was cleaved with *Bgl*II, the 5' termini were ³²P-labelled and the 431-bp *Bgl*II-*Pst*I fragment (Fig. 1C) covering the promoter region was isolated. S_1 mapping was as described in Fig. 3 legend.

of the β -globin promoter and the IFN- α_1 transcription unit contained more IFN- α_1 transcripts before induction than 11 h after. We conclude that IFN- α_1 RNA is also not stabilized by induction, and that its accumulation is due mainly to transcriptional activation. In drawing these conclusions we assume that the transcripts of the hybrid genes have the same stabilities as natural β -globin and IFN- α_1 mRNAs, even though their structures differ slightly at the 5' termini.

Our experiments further suggest that β -globin and IFN- α_1 RNA are degraded at different rates in L cells. This conclusion is based on the assumption that the promoter determines the same rate of transcription whether it is linked to a β -globin or an IFN- α_1 transcription unit, and on the finding that with either promoter, the level of the β -globin RNA is 10–100 times higher than that of the IFN- α_1 RNA. A considerable difference in the half lives of β -globin mRNA (90 h in erythroleukaemic cells)¹⁷ and of IFN- β mRNA (30 min in human fibroblast cells³) has been found *in vivo*, albeit in different cells.

The significance of the 5'-flanking regions of a gene for transcriptional control has been shown for several other genes. Fusion of the 5'-flanking region of a growth hormone, metallothionein or heat shock gene to the HSV *tk* transcription unit yielded hybrid genes inducible by glucocorticoids¹⁸, cadmium ion¹⁹ or heat²⁰, respectively. Similarly, the 5'-flanking region of mouse mammary tumour virus DNA rendered a dihydrofolate reductase transcription unit responsive to glucocorticoids²¹. In contrast, Pitha and her colleagues^{22,23} concluded that the elements essential for IFN- β induction lie within the coding or 3' noncoding region; these results require independent confirmation.

Which parts of the 5'-flanking sequence of the IFN- α_1 gene are required for transcription? We have shown that the ATA box is essential for β -globin transcription from the hybrid gene, and that the transcripts initiate 30 ± 3 bp downstream of the ATA box and not at the cap site of the β -globin gene. These

results confirm the role generally attributed to the ATA box, namely that of determining the transcription initiation site and sustaining maximal transcription of many (but not all) genes^{9,24,25}. In the accompanying paper²⁶ we have shown that deletions removing all but 117 nucleotides of the 5'-flanking region of the IN- α_1 gene do not abolish inducible transcription. Therefore, a DNA segment extending from position -117 to -6 suffices for inducible transcription. Further insights may be obtained by testing hybrid promoters, composed of elements from the IFN- α and the β -globin 5'-flanking sequence.

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1. Stewart, W. II. *The Interferon System* (Springer, New York, 1979).
2. Berg, K. *Acta path. microbiol. immun. scand. Sect. C, Suppl.* 279 (1982).
3. Raj, N. & Pitha, P. *Proc. natn. Acad. Sci. U.S.A.* **78**, 7426-7430 (1981).
4. Cavaliere, R., Havell, E., Vilecek, J. & Pestka, S. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4415-4419 (1977).
5. Sehgal, P., Dobberstein, B. & Tamm, I. *Proc. natn. Acad. Sci. U.S.A.* **74**, 3409-3413 (1977).
6. Morser, J. et al. *J. gen. Virol.* **44**, 231-234 (1979).
7. Mantei, N. & Weissmann, C. *Nature* **297**, 128-132 (1982).
8. Dierks, P., van Ooyen, A., Mantei, N. & Weissmann, C. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1411-1415 (1981).
9. Dierks, P. et al. *Cell* **32**, 695-706 (1983).
10. Berk, A. J. & Sharp, P. A. *Cell* **12**, 721-732 (1977).
11. Weaver, R. & Weissmann, C. *Nucleic Acids Res.* **7**, 1175-1193 (1979).
12. Mantei, N., Boll, W. & Weissmann, C. *Nature* **281**, 40-46 (1979).
13. Grosveld, G., de Bower, E., Shewmaker, C. K. & Flavell, R. A. *Nature* **295**, 120-126 (1982).
14. Shaw, G. D. et al. *Nucleic Acids Res.* **11**, 555-573 (1983).

15. Wilson, D. E. *J. Virol.* **2**, 1-6 (1968).
16. Thacore, H. & Youngner, J. S. *J. Virol.* **6**, 42-48 (1970).
17. Vollock, V. & Housman, D. *Cell* **23**, 509-514 (1981).
18. Robins, D., Peacock, I., Seeburg, P. & Axel, R. *Cell* **29**, 623-631 (1982).
19. Majo, K., Warren, R. & Palmiter, R. *Cell* **29**, 99-108 (1982).
20. Pelham, H. R. B. *Cell* **30**, 517-528 (1982).
21. Lee, F., Mulligan, R., Berg, P. & Ringold, G. *Nature* **294**, 228-232 (1981).
22. Reyes, G. et al. *Nature* **297**, 597-601 (1982).
23. Pitha, P. et al. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4337-4341 (1982).
24. Grosschedl, R. & Birnstiel, M. L. *Proc. natn. Acad. Sci. U.S.A.* **77**, 1432-1436 (1980).
25. Benoist, C. & Chambon, P. *Nature* **290**, 304-310 (1981).
26. Ragg, H. & Weissmann, C. *Nature* **303**, 439-442 (1983).
27. Nagata, S., Mantei, N. & Weissmann, C. *Nature* **287**, 401-408 (1980).
28. Chalberg, M. & Englund, P. *Meth. Enzym.* **65**, 39-43 (1980).
29. van Ooyen, A., van den Berg, J., Mantei, N. & Weissmann, C. *Science* **206**, 337-344 (1979).
30. Grunstein, D. & Hogness, D. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3961-3965 (1975).
31. Maxam, A. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* **74**, 560-564 (1977).
32. Wilkie, N. et al. *Nucleic Acids Res.* **7**, 859-877 (1979).
33. Aviv, H. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1408-1412 (1972).
34. Bernardi, G. *Meth. Enzym.* **21**, 95-147 (1971).

Polyoma virus 'hexamer' tubes consist of paired pentamers

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The discovery that the 72 capsomeres of the icosahedrally symmetric polyoma virus capsid are all pentamers¹ shows that the expected quasi-equivalent bonding specificity² is not conserved in the assembly of this virus coat protein. Tubular particles produced by polyoma and other papovaviruses seem to be polymorphic aggregates of capsomeres³ that may arise through variation in switching of the bonding specificity. Electron micrographs of wide and narrow classes of tubes were analysed by Kiselev and Klug⁴ using optical diffraction and optical filtering methods. The wide type were called 'hexamer' tubes because they consist of approximately hexagonally arrayed capsomeres that were assumed to be hexamers⁵, in accord with the quasi-equivalence theory of icosahedral virus particle construction². The narrow type were called 'pentamer' tubes because the capsomeres are arrayed in a particular 'pentagonal tessellation' which arises from the pairing of pentamers across 2-fold axes of the surface lattice⁴. Our reexamination of negatively-stained polyoma virus tubes by digital image processing of low-irradiation electron micrographs shows that all tubes are assemblies of paired pentameric capsomeres. We report here that the packing arrangement of the pentamers in the hexamer tubes is simply related to the pentagonal tessellation⁴ representing the packing in the narrow pentamer tubes. In all the tube structures examined, at least one pairwise contact between neighbouring pentamers closely resembles the contact between the pentavalent and hexavalent capsomeres in the icosahedral capsid¹.

Specimens were prepared from polyoma virus fractions sedimenting between the virion and capsid peaks on a sucrose density gradient. Electron micrographs of the negatively-stained specimens show the order of one tubular particle for every 100 icosahedral particles (diameter 450-500 Å). The most frequent tube structures (Fig. 1A) have a diameter of 400-450 Å and are built of approximately hexagonally packed capsomeres arrayed with one of the lattice lines at a small angle to the particle axis. Similar hexagonal packing is observed in tubes of diameter ranging from 275 to 525 Å. Micrographs of shadowed specimens show that the tubes are flattened and the near axial rows of capsomeres are inclined to the left, thus defining the hand of the surface lattices. About 10% of the tubes are the narrow (~300 Å diameter) pentamer type⁴.

Diffraction patterns were computed from images of minimally irradiated (<20 e⁻ Å⁻²) specimens⁵, negatively stained with 1% uranyl acetate. Figure 1B is representative of the hexamer tube diffraction patterns. The patterns from the front and back sides are related by approximate mirror symmetry about the meridional axis. Twelve pairs of dominant spots due to diffraction from the top hexagonal array of capsomeres (away from the grid as established from shadowed specimens) are marked by circles in Fig. 1C. This indexing, which is the same as that defined by Kiselev and Klug⁴, omits six additional spots, marked by squares, located at positions corresponding to a repeating unit of twice the area of the simple hexagonal unit cell. The intensity of these additional spots is sensitive to irradiation, although some are still evident even after exposure to >100 e⁻ Å⁻². For example, in the first analysed diffraction pattern from a polyoma virus hexamer tube⁴ (from a micrograph recorded with conventional high irradiation) one strong and several weak spots from the larger unit cell can be discerned, as we now know where to look. Our scheme for indexing the hexamer tube diffraction pattern identifies a near orthogonal unit cell. From the average of measurements on 18 flattened hexamer tubes, the cell dimensions for the front surface are 89 × 137 Å subtending an angle of 83° and the cell is oriented with the short axis at ~45° to the right of the tube axis; for

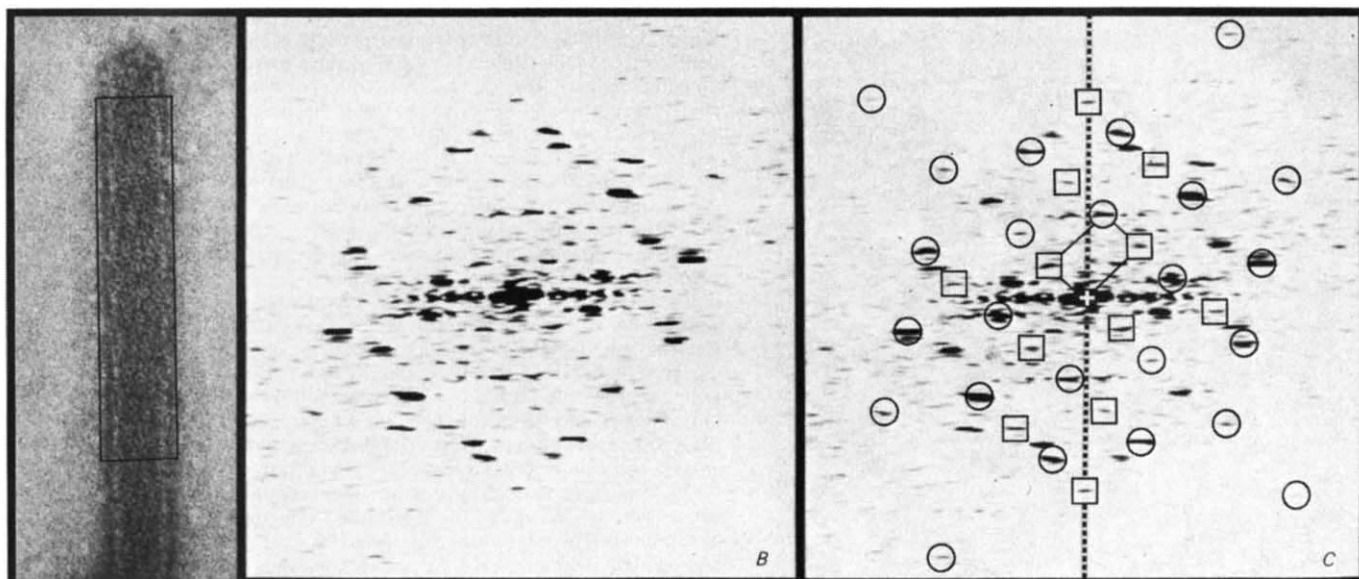


Fig. 1 Indexing of the diffraction pattern from a polyoma virus hexamer tube image shows that the true unit cell is twice as large as the simple hexagonal cell previously described⁴. The electron micrograph (A) is representative of the most frequent class of hexamer tube. The tube, capped at both ends, appears uniformly flattened except where it is supported by the adjacent icosahedral capsid. Superposition of the front and back lattices gives rise to a characteristic criss-cross pattern of near axial rows of capsomeres. Scale bar, 500 Å. The diffraction pattern computed from the area boxed in A is shown in B and again in C with indexing of the spots arising from the top layer (away from the grid) of the flattened tube. Circles indicate spots from the simple hexagonal unit cell, which would contain only one capsomere, and the squares mark additional spots from the repeating unit of double this size. Spots from the bottom side (not marked) lie on a lattice related to that from the top by approximate mirror symmetry about the meridional axis (dashed line). The reciprocal lattice unit cell for the top layer is marked at the centre of the pattern.

the back surface (in contact with the support film) the dimensions of the approximately mirror symmetric cell are 95×147 Å, subtending an angle of 89° . The shrinkage of the top surface relative to the bottom is typical for negatively-stained specimens⁶.

The filtered image (Fig. 2A), computed for the front layer using the 18 pairs of spots indexed in Fig. 1C, shows the packing arrangement of the pair of capsomeres in the unit cell. The capsomeres appear pentagonally shaped in the clearest parts of the filtered image and are arranged in a surface lattice having p2 symmetry. Models built of regularly packed, pentagonally symmetric units have been constructed to fit the features seen in the filtered micrographs. A computer-generated image of the front view of the planar model (with lattice dimension corresponding to the upper layer of the flattened tube) is compared with the filtered electron micrograph in Fig. 2B.

The relation of the pentamer packing in the hexamer tubes to that in the pentamer tubes⁴ is illustrated in Fig. 3. Common to both is a zigzag ribbon arrangement of pentamers connected by adjacent edge-to-edge contacts; this ribbon motif is inclined at an angle of $\sim 45^\circ$ up to the right of the tube axis. Within a ribbon in the tube surface, the adjacent edge-to-edge dimer contacts are of two different types: one indicated by the nearly vertical 'zig' lines in Fig. 3 between the pentamer pairs oriented in the axial direction, and the other by the nearly horizontal 'zag' lines between the pentamer pairs in the direction of maximum curvature. Each pentamer in the zigzag ribbons of the pentamer tube tessellation⁴ (Fig. 3B) makes a third type of edge-to-edge dimer contact in a diagonal direction (inclined up to the left) with a pentamer in a neighbouring ribbon. In the hexamer tube surface lattice (Fig. 3A), the ribbons are shifted so that each pentamer makes a short edge-overlap dimer contact and a vertex-to-vertex pairing with two pentamers in an adjacent ribbon. The directions of these two types of inter-ribbon contacts in the hexamer tube lattice are nearly parallel to the zig and zag lines in Fig. 3A. This hexagonally coordinated arrangement of pentamers produces a pattern of unequally spaced pairs of small triangular gaps

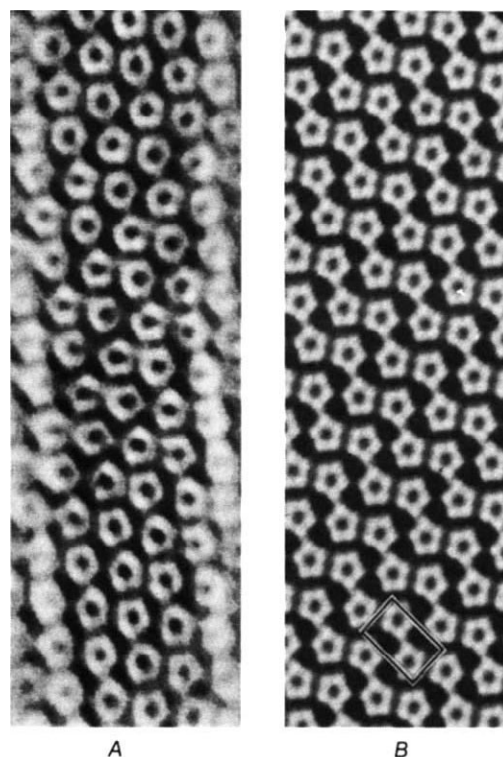


Fig. 2 The filtered image of the front layer of a flattened hexamer tube corresponds to a model surface lattice built of pairs of pentamers arrayed with p2 symmetry. Image A was computed from the diffraction pattern of the portion of the tube boxed in Fig. 1A. The pattern was filtered to include the spots indexed in Fig. 1C and the equator (with the equator and the meridional pair of spots given half weighting). The planar model (B) was built in the computer from regular pentamers arranged in a p2 lattice at the coordinates measured for the capsomeres in the unit cell of the filtered image. One choice of unit cell is boxed.

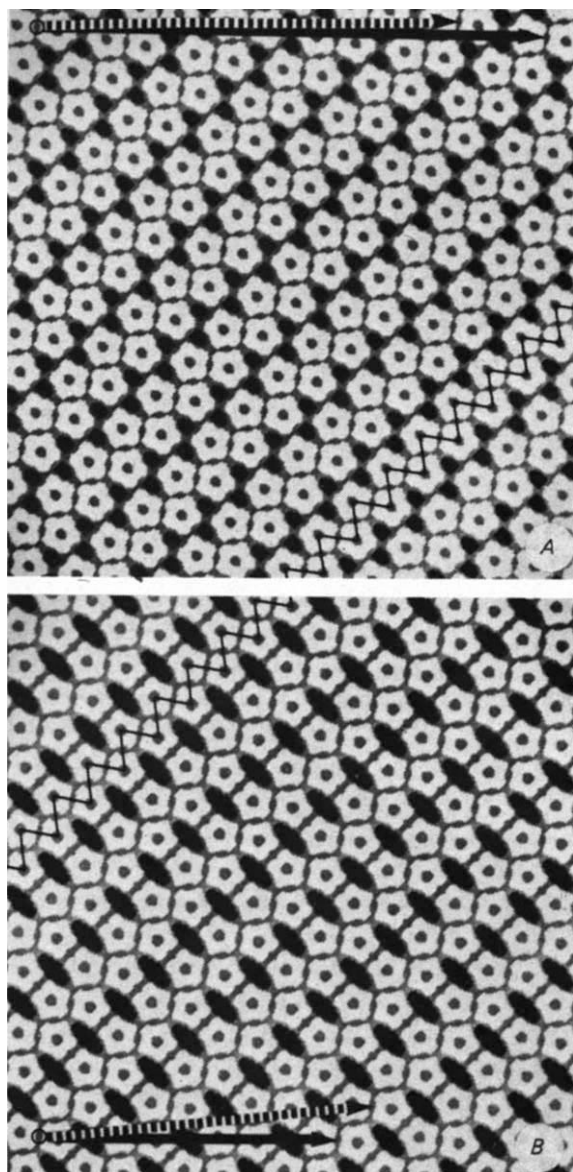


Fig. 3 Comparison of the pentamer packing in hexamer (A) and pentamer (B) tube surface lattices illustrates correspondences in bonding contacts. The 'zig' and 'zag' lines (oriented respectively nearly vertically and horizontally along the diagonally directed ribbon) indicate the two classes of dimer 'bonds' between pentamers that are conserved in the hexamer and pentamer tube lattices. These planar models were constructed with equivalent connections for the differently oriented edge-to-edge bonds between the pentagonal units, and the packing width of the zigzag ribbon in A was made the same as in B. In the cylindrically curved tube surface, the differently oriented edge-to-edge contacts are non-equivalently bent. Furthermore, the relative separations of capsomeres in the different bonding directions measured from electron micrographs are slightly distorted compared to these idealized models. Circumferential vectors corresponding to different tube structures are marked by arrows between equivalent lattice points. Models of the tubes can be constructed from these plane lattices by cutting out rectangles based on the circumferential vectors and connecting the vertical edges to form cylinders. Two of the frequently observed size of hexamer tubes are indicated by the arrows at the top of A, the dashed one representing the circumference of the tube in Fig. 1A. Hexamer tubes of larger and smaller diameter have circumferential vectors in approximately the same direction as the marked arrows. The circumferential vectors of the zero- and one-start pentamer tubes⁴ (which are the only identified narrow tubes of this type) are marked by the solid and dashed arrows, respectively, at the bottom of B.

between ribbons. Alignment of the ribbons in the pentamer tube lattice to form the diagonal edge-to-edge contacts (Fig. 3B) generates the distinctive pattern of large lozenge-shaped gaps that is a dominant feature of the electron microscope images⁴.

All the polymorphic tubular assemblies of polyoma virus capsomeres that we have analysed are constructed from paired pentamers. A rare class of wide tube, to be described elsewhere, is built of edge-to-edge bonded pentamer dimers oriented alternately parallel and perpendicular to the tube axis in an approximately square surface lattice. The curvature of all the tubular surface lattices requires bending at the edge-to-edge connection between the pentamer pairs oriented in the circumferential direction. We infer that this bent contact between pentamers in the wide hexamer tubes is similar to the edge-to-edge connection between the pentavalent and hexavalent pentamers in the capsid¹, whose axes subtend an angle of $\sim 25^\circ$; greater bending

of the circumferentially oriented pentamer pairs is needed to form the narrow pentamer tubes. The two other types of contacts identified between pairs of hexavalent pentamers in the capsid¹ are similar to the two kinds of dimer contacts between pentamers of adjacent zigzag ribbons in the hexamer tubes. Thus, the polymorphism of the tubular aggregates correlates with the variable bonding potential of the pentameric capsomeres evident in the icosahedral capsid.

The quasi-equivalence theory of icosahedral virus particle construction² required hexamers as well as pentamers of the protein subunits to conserve bonding specificity. Previous observations on the structure of papovavirus capsids and their polymorphic variants were believed to be consistent with this theory. In retrospect, there was no compelling experimental evidence to support the belief in the existence of hexameric capsomeres in papovaviruses. Our recent X-ray diffraction^{1,7,8} and electron microscopy studies are compatible with the previous observations and show that all polyoma virus capsomeres are in fact pentamers. The logic of the all-pentamer assembly may be explained by a capsomere model with switchable bonding specificity that can build the icosahedral capsid and the polymorphic tubes.

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1. Rayment, I., Baker, T. S., Caspar, D. L. D. & Murakami, W. T. *Nature* **295**, 110–115 (1982).
2. Caspar, D. L. D. & Klug, A. *Cold Spring Harb. Symp. quant. Biol.* **27**, 1–24 (1962).
3. Finch, J. T. & Klug, A. *J. molec. Biol.* **13**, 1–12 (1965).
4. Kiselev, N. A. & Klug, A. *J. molec. Biol.* **40**, 155–171 (1969).
5. Baker, T. S. & Amos, L. A. *J. molec. Biol.* **123**, 89–106 (1978).
6. Moody, M. F. *J. molec. Biol.* **25**, 167–200 (1967).
7. Rayment, I. *Acta crystallogr. A* **39**, 102–116 (1983).
8. Rayment, I., Baker, T. S. & Caspar, D. L. D. *Acta crystallogr. B* **39** (1983).

Big problems for archaeology

Clive Gamble

In Pursuit of the Past: Decoding the Archaeological Record.

By Lewis R. Binford.

Thames & Hudson: 1983. Pp.251. £12.50, \$18.50.

ARCHAEOLOGISTS tell the oldest tales of all. In many cases they have been recounting the same stories for the past 150 years, give or take a new discovery like the footprints at Laetoli or a breakthrough such as radio-carbon dating. While these embellishments may have altered the punch-lines, the way that interpretations of life in the past are constructed have a very comfortable and entirely familiar ring to them. The past, we are told, is another country which we have done well to leave behind. The mastery of nature by mankind was a slow process that apparently required the development of human intelligence and its application to technology and economic resources. As a result many archaeologists have accepted the challenge of the subject only by adding discoveries to the pile of data that now exists, rather than in critically examining the way these threads are woven into the impressionistic tapestries that we have made of antiquity.

During the past two decades a great many alternative interpretations of the past have been put forward. These have been exciting times for the discipline as field-work commenced in the largely untouched philosophical, theoretical and epistemological areas of the subject. This has led to the production of memorable anecdote and parable by those privileged to have lived through such heady days. However there has been little apparent impact on a wider audience who, if they had been allowed into the stadium, would have been bemused by the antics on the pitch.

If archaeology is truly in a state of fundamental change, as many would contend, then what indications should we see? How can the spectators be brought into the action and so appreciate that something other than competition amongst academics is taking place?

One set of answers is vividly illustrated in this book by Lewis Binford, who, during the past 20 years, has played in most positions on the field, in the stands and on the bench. He may not have blown the whistle to get the new game started, but he certainly kicked the first field-goal in 1962 with a short paper entitled "Archaeology as Anthropology" and since then has been instrumental in rewriting the rule book.

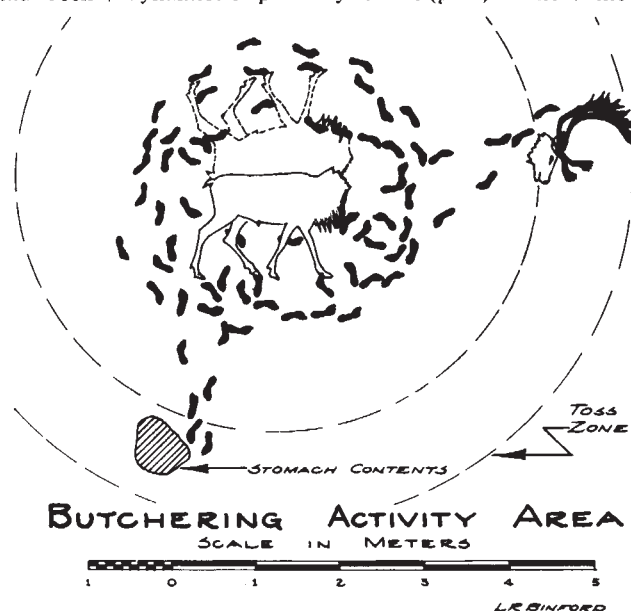
Binford's position, if not his tactics, has remained constant during this period of archaeological upheaval, as he recounts in a biographical sketch in Chapter 5. His major contention is that archaeologists have underestimated the complexity and variability of the archaeological record and have undervalued its potential for investigating patterns of human behaviour. They have simplified rather than enriched our understanding of past human behaviour by cossetting their discoveries in safe, preconceived categories. For example, past societies have been labelled as big-game hunter, maize agriculturalist or proto-urban without any deeper appreciation of how and why the different archaeological records were created. In this regard the past has been an under-used resource for the study of variability in human behaviour.

The central problem, addressed by Binford over many years, is how to unlock this potential. The main obstacle, in his view, lies not in the limitations of a piecemeal and worm-eaten data base but rather in our methodological naivety. Much of his recent research has been directed towards decoding the archaeological record through the construction of bridging arguments (middle range theory). In his words "the challenge that archaeology offers . . . is to take contemporary observations of static material things and, quite literally, translate them into statements about the dynamics of past ways of life (p.20)". The

book clearly demonstrates that if archaeology is to progress as a discipline then this enormous challenge must be tackled. To do so requires a re-examination of basic principles which deal with the formation of the archaeological record, and thus a prime concern must be with epistemology.

How the challenge can be pursued is set out in the second section of the book, where the question "What does it mean?" is addressed. Here Binford recounts work in progress with some results of his ethno-archaeological research among the Nunamiut Eskimo. He details the use of space and territory by this hunting group at a variety of scales. In particular Binford approaches the analysis of camp sites by documenting the way that patterns in material objects are created on the surface of the ground by such everyday activities as sleeping, eating, cooking, mending tools and watching for caribou. Work space and seating plans produce very strong circular patterns of refuse around fixed features such as hearths. The many excellent photographs and plans of the Nunamiut and other hunter-gatherer camps illustrate the regular and repeated patterns. These patterns result from constraints such as the size of the human body, wind direction around an open hearth and whether an activity is carried out in a sitting or standing position. Once understood, contemporary observations on how patterns are created as a by-product of behaviour act as a yardstick for interpreting a set of static observations within the archaeological record. This is demonstrated by the re-interpretation of a number of well-known palaeolithic camp sites. The return to very basic properties of the archaeological record highlights the extent to which archaeologists have previously only assumed they understood the meaning of the patterns they spotted in their data.

The other two sections in the book provide more examples of similar approaches which archaeologists have used to answer the questions "What was it like?" and "Why did it happen?". The first summarizes the arguments in Binford's book *Bones: Ancient Men and Modern Myths* (Academic, 1981 — for review see *Nature* 295, 82; 1982) which uses inferences drawn from the observations of patterns of bone and animal carcass destruction by both carnivores and man to question our view of early hominids as mighty hunters. The final section finds Binford putting forward his own ways of accounting for the origins of agriculture and the rise of complex society. These are two of archaeology's Big Questions, and the models he presents are characteristic challenges to accepted views that environments



A hunter-gatherer quickstep — how patterns are created for the archaeologist to decode, here the activity area used by the Nunamiut to butcher one caribou. (From *In Pursuit of the Past*.)

rich in food and the effects of trade goods had a large hand in the two processes. His own explanatory sketches are a way of saying "I have as good if not better imagination than the next archaeologist; so believe my story!" — except that he continues to throw down those challenges to develop methodology in order to make reliable inferences rather than inspired judgements on the archaeological record. Although Binford has concentrated his research among hunters and gatherers, these forays into later prehistory point out very neatly that archaeological inference does not get easier as life became more complex and hence closer in appearance to our own cultural experience.

Many archaeologists have been hoping that Binford would write an accessible book of this nature. With the assistance of the collaborative editors, John Cherry and Robin Torrence, this text has captured one man's enthusiasm for his research as it was delivered in lectures in England and the Netherlands. It carries his classic stylistic trademarks including some coruscating footnotes, unmistakable line drawings, new additions to archaeological vocabulary and above all a willingness to confront archaeology's big problems head on. No one can read this book without acknowledging that archaeology is finally digging up its nineteenth century roots and burning them. Lewis Binford shows, by example, the strategies that are needed to turn the subject from a backward-looking curiosity into a scientific attack on our understanding of past human behaviour. □

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Journals' review issue 1983

On October 6th *Nature* will publish the third review supplement devoted to science journals. The two previous supplements (covering journals first published between January 1978 and May 1980, and June 1980 and May 1981) appeared in *Nature* 293, 341–369 (1981) and 299, 491–514 (1982).

Criteria for inclusion of a journal in the 1983 issue are that:

- the first number appeared, or the journal was re-titled, between June 1981 and May 1982;
- it is published at least three times a year;
- the main language used is English.

Broadly, periodicals of professional interest to scientists will be considered for review, with the exception of abstracts' journals. Journals appearing prior to June 1981 but after January 1978, and not covered in the previous issues, will also be considered.

Publishers and societies are invited to submit four sample issues of journals satisfying the above criteria, including the first and most recent numbers, to the Review Editor, *Nature*, 4 Little Essex St, London WC2R 3LF, England.

Condensed matters for the expert

P.N. Butcher

The Structure and Properties of Matter.

Edited by T. Matsubara.

Springer-Verlag: 1983. Pp.432.

DM100, \$40.

It is a brave author who sets out in the last quarter of the twentieth century to describe the modern theory of condensed matter from a unified point of view. He needs help. He needs skill in selecting which topics to include and which to leave out. Above all he needs to define his audience carefully. Failure on any of these counts will mean either that the work is never finished or that no one will read it when it is.

With the expert help of Professors Tsuneto, Murao, Matsuda and Yonesawa, Professor Matsubara has successfully avoided all these traps for the unwary. He and his co-authors present essentially simple but tightly knit arguments which start from the first principles of quantum and statistical mechanics and gently guide the reader towards the modern view of matter. In the beginning there are electrons and nuclei. At the end there are metals, semiconductors, superfluids, ferromagnetics, crystals and glasses. It is a fascinating journey but not one to be undertaken lightly by the reader. He needs to keep his wits about him to see all the navigational aids provided as they flash rapidly past in the brief span of 432 pages.

Generality must be the keynote in a book like this and it is admirably maintained throughout. Particular materials and experiments are described only when they illustrate the theoretical concepts which are the chief concern of the authors. For the most part these are the ideas which occupied the centre of the stage when the first edition appeared in Japan in 1973: band structure, superfluids, magnetic materials, the Kondo effect and disordered systems. The present volume is a translation of a revised edition published in 1978.

The book is written in a simple style by experts. It is intended to be read for pleasure by experts, and they will enjoy the succinct way in which the authors handle the intricate problems which they take up. Students should look elsewhere. The treatment is very concise and the reference lists are short, most of the citations being to material published before 1975 which is already beginning to seem quite a long time ago. The weight given to different topics also reflects the fashions of the early 1970s. There are separate chapters on ^4He and ^3He and another on the Kondo effect. On the other hand superconductors get only ten pages and Anderson localization is given

just one page in a chapter on random systems.

The authors have rigorously excluded some topics in order to be able to write at reasonable length about others. Electron transport phenomena are the most regrettable omission. This has been a fruitful field over the past 15 years, both in Japan and elsewhere, and it is disappointing not to find some reference to it here. Less serious is the omission of treatments of X-ray, electron and neutron diffraction studies of atomic structure and of the thermal properties of condensed matter. These are readily available elsewhere. By leaving them out the authors have been able to write extensive accounts of both pseudopotential and tight-binding band structure methods, and of the coherent potential approximation, which are at once both excellent and timeless. □

P.N. Butcher is Professor of Theoretical Physics at the University of Warwick.

Reference: mine as a mountain

Alan Isaacs

Van Nostrand's Scientific Encyclopedia, 6th Edn.

Edited by Douglas M. Considine.

Van Nostrand Reinhold: 1983. Pp.3,067.

\$107.50, £95.

THE reappearance of *Van Nostrand's Scientific Encyclopedia* in a new edition (the sixth) only seven years after the last edition, combined with its sheer bulk (3,067 pages, 700 more than the fifth edition), provides welcome reassurance that this very considerable work is now a permanent feature of the scientific landscape — the Mont Blanc, if not the Everest (the 15-volume *McGraw-Hill Encyclopedia of Science and Technology* is surely that), of scientific encyclopaedias.

Because I do not believe that a reviewer is entitled to denigrate in a few brash sentences a work that has taken a number of people several years to produce, I would not say that this encyclopaedia was awful even if I thought so. As it happens, the book is undoubtedly a mine of very useful information, particularly in the newer and more exciting areas of science and technology. Much of this information would be hard to unearth in such a concise form elsewhere. I do, however, have two reservations.

The first is that the book has a very heavy American bias, probably unnecessarily so. Technological examples, end-of-article bibliographies and illustrations of equipment are all almost exclusively American. Even if the American parish is the biggest and most influential in the whole scientific congregation, a reference work such as this

should not be parochial. An encyclopaedia of science needs to be — and to be seen to be — as ecumenical as it possibly can.

My second reservation is perhaps more serious. It also arises from parochialism. Scientists throughout the world have now embraced SI units as a part of their creed. The editor of *Van Nostrand's Encyclopedia* seems to regard this as a form of heresy with which he is prepared only to compromise. In his preface, he writes that this is the first edition of the work to include both metric and English units (whatever was he thinking of in 1976 and 1965?). Yet even now he has settled for cgs units in almost all cases. This has led him into error. The entry for heat, for example, refers with more reverence than comprehension to the "presently accepted value for the mechanical equivalent of heat", describing it (as if this was the new edition for the 1880s) as "an important constant". It should hardly be necessary to add that in SI units all quantities of heat and energy are expressed in joules and the mechanical equivalent of heat is reduced to a milestone of mild historical interest (and a conversion factor to get one out of obsolescent calories into main-stream joules).

This article on heat was not in fact commissioned for the new edition; but even as a contribution to the fifth edition its author should have known better. However, new articles written for the sixth edition are usually of a very high standard. It is difficult to select examples because, as the preface tells us, nearly 100 entirely new topics have been added, with over 7,300 new entries. These include automotive electronics (seven pages exclusively about American cars), cancer research, fuel cells, information theory, insecticide and pesticide technology (and the aspects of genetic engineering applicable to it), Mars, microelectronics, quarks, satellites, voice recognition and synthesis, and X-ray (CAT) scanners, to name but a few excellent review articles that illustrate the range of the book. Guaranteed to put you off your food (and certainly more effective than the F-plan diet) is the new 10-page article on foodborne diseases. Salmonellosis and botulism are familiar and frightening enough. But what about hymenolepiasis diminuta and the hazards arising from contaminated popsicles?

Even if you are not American and even if you think that all physical quantities should be expressed in SI units, this encyclopaedia is worth having on your shelf — provided, of course, that the shelf is very, very strong (the book weighs 14 pounds — excuse me, it has a mass of 6.35 kilograms and a weight of 62.27 newtons).

Alan Isaacs is editor of the Macmillan Encyclopedia, the Penguin Dictionary of Science, Longman's New Dictionary of Physics, and science editor of Collins English Dictionary. He has contributed to many other dictionaries and encyclopaedias.

Organs to molecules: the new chemistry of disease

D.J. Weatherall

Biochemical Aspects of Human Disease.
Edited by R.S. Elkeles and A.S. Tavill.
Blackwell Scientific/Blackwell Mosby:
1983. Pp.730. £49.50, \$95.

THOSE who are just starting their careers in clinical medicine don't know how lucky they are. Despite the gloomy prognostications of MacFarlane Burnet and others in the early 1970s, remarkable advances in molecular and cell biology are starting to have a great impact on our understanding of disease mechanisms. All the signs are that the clinical sciences are about to enter the most exciting period of their development.

However, the rapid change in emphasis from whole-organ to cell and molecular pathology is causing major problems for many clinicians. Those who qualified more than a few years ago do not have the basic training required to appreciate the potential implications of these developments, while those who are currently going through their medical training are not always much better off. Many pre-clinical courses still make very little effort to point out the relevance of the basic sciences to clinical practice. If clinicians are to make the best use of what the basic sciences have to offer, we shall have to do some rethinking about the organization of clinical academic departments, and create a new breed of teacher who can somehow keep a foot in both camps. Furthermore, the information flow will have to be in both directions; many basic scientists have little idea of the potential value of their work for the clinical world or are very naive when it comes to applying their technology to medical problems.

With these thoughts in mind I was encouraged to read on the dust cover of this new book that it is designed to highlight recent advances in our understanding of the mechanisms of disease at the molecular level. In the preface the editors make it clear that they have not attempted to be comprehensive but rather have selected areas in which "major new frontiers have been established by recent research advances". Hence, this should be an exciting and timely book. Though in many ways it is, I don't think that it has been entirely successful in catching the real fascination of molecular medicine.

A broad range of topics is covered including the mechanisms of hormone action, diabetes, lipid disorders, muscle disorders, calcium metabolism, gastrointestinal and liver disease, porphyrin metabolism, the nervous system, psychoses, drug reactions, hypertension

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by Irene A. McCulloch, PhD

This monographic work, in preparation for more than 30 years, consists of three parts on the Pacific, totaling more than 1000 pages with 200 folio-sized plates and covering some 360 genera and 2,300 species. Part IV on the Atlantic consists of 361 pages with 72 folio-sized plates covering more than 700 taxa. The folio-sized volumes are on 50% rag paper, bound in durable dark red Buksyn with gold stamped covers.

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and inflammation. The problem is that some of these accounts are lacking in focus. For example, the sections on liver disease, muscle disease, hypertension and clinical pharmacology, while comprehensive, consist of material which can be found in most standard textbooks. There is no particular effort to define those aspects which are, or might be, particularly amenable to the newer techniques of cell or molecular biology.

Again, the well-written haematology section gives a good account of the structure and function of haemoglobin, sickling disorders and red cell enzyme defects, but says little about the wealth of information that has been obtained in the last few years by cloning and sequencing the globin genes, an area of research which has probably told us more about human molecular pathology than any other. Despite the remarkable advances in our understanding of the workings of the immunoglobulin genes and of the development of the immune system, the only chapter on immunology deals with autoantibodies and disease, a good account but an odd choice for a book that sets out to highlight recent advances in molecular aspects of medicine. The book contains no mention of malignant disease, despite the recent achievements in the fields of oncogenes and surface markers. Other important areas of great potential for clinical application, including membranes, the

vascular endothelium and the chemistry of platelets and clotting factors, get scant mention or do not appear at all. In particular, it is disappointing to see a book on molecular mechanisms of disease appear in 1983 without a simple account for the non-specialist of the methods of recombinant DNA technology which promise to revolutionize the field over the next few years.

This book gives a competent and in some parts very good account of the chemistry of selected groups of human diseases. Where it seems to fall short of its aims, at least to me, is in its failure to highlight some of the particularly active areas of cell and molecular biology which are starting to make a

real impact on our understanding of molecular pathology. Could it be that it had too long a gestation time — there are very few references later than 1980? Perhaps the cumbersome process of producing a large, multi-author textbook is not compatible with catching the excitement of a field which is moving so fast. This is an important problem for authors and publishers alike. The information gap between clinicians and basic scientists is widening rapidly; both will lose out if it can't be bridged. □

D.J. Weatherall is Nuffield Professor of Clinical Medicine at the University of Oxford.

Mixed bag of cells

John Scholes

Cell Biology of the Eye.

Edited by David S. McDevitt.

Academic: 1982. Pp.570. £50, \$75.

TO ACHIEVE transparency, the eye chamber is sparsely populated by cells and so at face value of unlikely interest to cell biologists. However, the cornea and eye media contain abundant secreted matrix proteins and glycosaminoglycans, thought to be important generally in the formation and maintenance of tissues but usually inaccessible on account of their sparse interstitial distribution. Moreover, the lens substance comprises a family of stable proteins, the crystallins, purified almost to homogeneity by systematic cell autolysis during development. These biochemist's lures explain why the cell biology here has much less to do with any visual processes than with the inert media traversed by light on the way to the rods and cones.

Accordingly the first seven of the eleven chapters in *Cell Biology of the Eye* concern the cornea and lens. Among these, G. W. Hart reviews proteoglycans of the corneal stroma. *Inter alia*, the proteoglycans seem to shape the pattern of collagen fibrillogenesis, and this could be a valuable prototype for explaining the properties of more elusive matrix structures elsewhere. D. Gospodarowicz and L. Giguere deal with interactions between corneal cells and the extracellular matrix. In their laboratory, corneal cells have provided a definitive test-bed for the ideas that matrix factors are permissive for cell proliferation and foster epithelial growth.

The contributions dealing with the lens necessarily emphasize cellular rather than extracellular features. D.S. McDevitt, the editor, describes with S.K. Brahma immunocytochemical aspects of lens development, while T. Yamada reviews intriguing parallels between Wolffian lens regeneration *in vivo* and the trans-differentiation, seen in culture, of various eye cells to manufacture lens protein.

These reveal a peculiar leakiness in the crystallin gene cluster, but have not yet cast general light on mechanisms controlling gene expression.

Just four chapters at the end of the book deal with the retinal interface between light and sight. This means the coverage, if interesting, is anything but comprehensive. Perhaps neglected topics were deliberately chosen. Thus, one of the best-understood functions of the vitamin A family of compounds is surely as chromophores in vision, the main purpose of the eye. However, G. J. Chader reviews their pleiotropic roles elsewhere in the organism, emphasizing co-factor and hormone-like properties. What better introduction could this stimulating chapter have formed to another which is conspicuously lacking? The absent friend is the fast-moving cell biological topic of the enzymatic cascade between rhodopsin and cytoplasmic nucleotide messengers in retinal rods. This is dealt with only in passing by D. S. Papermaster and B. Schneider, who primarily review photoreceptor membrane assembly processes. Otherwise, there is a thoughtful chapter by R. E. Marc on the distribution of retinal cone cell types, and W. Morgan reviews aspects of retinal dopaminergic function.

It was enjoyable excavating this mixed bag free of charge, but much of the material could have been unearthed elsewhere, sometimes in more up-to-date form. Much was invested in the inert and transparent functions of the eye, at the expense of the retina. This emphasis could have been a deliberate choice, but other signs suggest editorial perfunctoriness, not least the abundant typographical errors which sometimes generate absurdities of meaning. □

John Scholes is at MRC Cell Biophysics Unit, King's College, University of London.

● **Darwin and Huxley.** Next week Oxford University Press issue a paperback edition of *Charles Darwin and T.H. Huxley: Autobiographies*, a book first published in 1974. Both "autobiographies" are little more than sketches, but the book is rounded out by a bibliography, chronologies and by an introduction by Sir Gavin de Beer. Price is £2.50.

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American Society of Biological Chemists Meeting

This week's range of products includes items being exhibited at the meeting of the American Society of Biological Chemists which will be held in San Francisco from 5 to 9 June

Biologicals

● A chemically synthesized gene coding for human pancreatic growth hormone releasing factor (hpGRF) is now available from Creative BioMolecules. This peptide will direct the secretion of growth hormone in humans and may be useful as a treatment for certain human growth disorders. Creative BioMolecules is offering the synthetic hpGRF gene on a nonexclusive basis to any university or industrial group interested in the study or production of hpGRF. The synthetic hpGRF gene was designed at Creative BioMolecules based on the 44 amino acid sequence of hpGRF, reported by Dr. Roger Guillemin and his colleagues (*Science* **218**, 585-587, 1982).
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● Boehringer Mannheim offers a new adsorbent which can be used to isolate plasma proteins such as α_2 -macroglobulin, α_1 -protease inhibitor or α_2 -human serum glycoprotein. The affinity adsorbent can also be used to isolate and characterize interferon from various sources and to separate nucleotides. The zinc chelate affinity adsorbent contains about 3% zinc (in chelate form) fixed through a spacer to agarose. This means that the affinity adsorbent can be regenerated by treating the carrier-material in the column with EDTA and zinc chloride-solution.
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● Hybritech Incorporated has released Tandem-E IgE, a solid phase, two-site immunoenzymetric assay (IEMA) for the quantitative measurement of immunoglobulin-E (IgE) in serum. Tandem-E IgE is a non-isotopic assay system which incorporates monoclonal antibody technology to produce a specific assay for IgE. Tandem-E IgE offers sensitivity to 2.4 IU per ml with a linear range to 300 IU per ml. It gives single point calibration and quantitative results are obtained in 3 hours using a standard spectrophotometer. The kit has a shelf life of one year.
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● Becton Dickinson Immunodiagnosics now have available a prolactin radioimmunoassay (RIA) kit. The kit offers a single incubation of 1½ hours at 37°C which means that results can be obtained for prolactins in 2 hours. The kit also has colour coded reagents.
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● Haem-C is a seven-parameter blood control from Baker Instruments. It is human blood, and is stabilized (not fixed) to closely mimic patient samples. Haem-C is available in 3 levels — normal, abnormally high and abnormally low.
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● Mirsky's fixative has been developed by National Diagnostics as a replacement for formaldehyde and glutaraldehyde. It contains no toxic buffers or formaldehyde. Mirsky's fixative is cross linking and double binding, which allows the determination of cellular morphological detail when the specimen is subjected to light and electron microscopy. Tissue shrinkage and brittleness are reduced.
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● Pharmacia have introduced two new products for density gradient centrifugation, for use particularly in nucleic acid research and virology. CsTFA is supplied in solution and contains 134 g of caesium trifluoroacetate per 100 ml of solution with a density of 2.0 g per ml (± 0.05). CsTFA can be used to separate nucleic acids and proteins by isopycnic centrifugation. It can also separate messenger, ribosomal and transfer RNA and will inhibit nuclease activity. In addition, optical and reagent grades of caesium chloride are available from Pharmacia in 50 g and 250 g packs.
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Pharmacia's centrifugation products

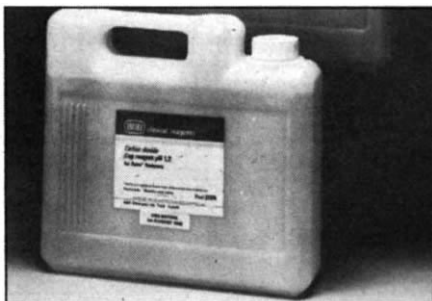
● NMS Pharmaceuticals has produced a new human chorionic gonadotropin (HCG) assay. The test gives a sensitivity of 2 mIU per ml at 90% binding. The screen assay uses a 10 or 25 mIU per ml cut-off and one incubation.
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● A reverse transcriptase system has been produced by Enzo Biochem (ERP-112) which consists of 100 units of avian myeloblastosis virus (AMV) reverse transcriptase, 500 units of human placental ribonuclease inhibitor, and 10 mg of bovine serum albumin (BSA).
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● Chemical Dynamics has produced L-2-oxothiazolidine-4-carboxylic acid, an amino acid analogue. In peptide synthesis, L-2-oxothiazolidine-4-carboxylic acid can act as an analogue of both thioproline and 5-oxoproline.
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● A programme to extract restriction enzymes from blue green algae is being conducted by Cambridge Biotechnology Laboratories. So far they have produced the enzyme Aha III from *Aphanethece halophytica* which cuts the TTT/AAA sequence and is designed to be used in plant, mitochondrial or recombinant DNA research.
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● E M Diagnostics, the diagnostics division of BDH Chemicals, has expanded its range of clinical assay reagents to include Beckman Astra Solutions. The solutions for creatinine, glucose and urea determinations are also suitable for use on the corresponding Beckman benchtop analysers.
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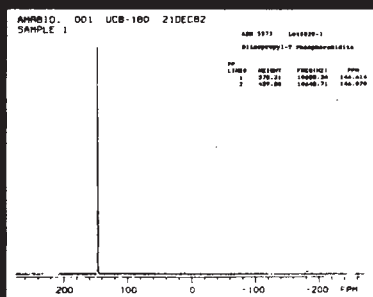


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General laboratory equipment

● Biotech Research Laboratories has introduced a new multichannel pipette tip cannister. The cannister will sterilize and store 96 pipette tips. The 96-well configuration allows access to the pipette tips in all configurations of 1, 4, 8 and 12 tips.

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● Cambridge Biotechnology Laboratories, in conjunction with the Howe Group Engineering Division, have produced a family of gradient formers for producing linear, convex, concave and exponential gradients for gel electrophoresis and gradient ultracentrifugation. Four gradient systems are available which offer total gradient volumes of 150, 250, 750 and 2,000 ml respectively.

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● A 12×75 mm sterile tube with a two position cap is available from Sarstedt. With the polyethylene cap in the closed but unsealed position (position 1), samples are maintained as aerobic for microbiological procedures. When the cap is in the sealed position (position 2), the tube can be used for laboratory storage, transfer, and centrifuge applications.

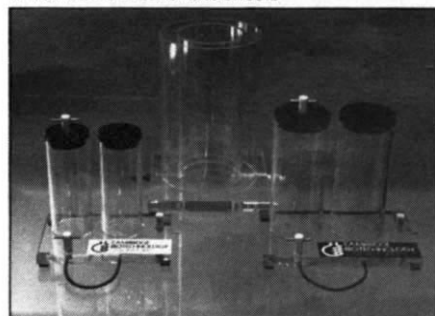
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● A new range of pH indicator papers is now being produced by Whatman Paper Division. The type SR (standard range) pH Indicator Papers provide colour changes over the full pH range of 1–14. Type TC pH indicator papers have three separate colour bands on a strip. Type CF pH indicator papers use plastic support strips and cover the range pH 0–14. With type CS papers, each test strip has its own printed colour comparison chart. They cover pH 1–12 in six narrow range packs and a single wide range strip. Also available are nitrocellulose membrane sheets for blot transfer and binding.

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Whatman's membrane sheets



Gradient formers for gel electrophoresis

● A disposable plastic vial, 9×23 mm, suitable for radioimmunoassay testing, has been developed by Sarstedt. The vial is small to keep waste volume to a minimum. A styrofoam storage which holds 96 tubes and an anodized aluminium rack are also available as accessories.

Circle No. 116 on Reader Service Card.

● The range of accessories available for the Microfuge benchtop centrifuges from Beckman has been increased by the addition of a fixed angle rotor. The new 12-place aluminium rotor holds tubes at a 45° angle, and top speeds of 13,250 r.p.m. (with the Microfuge 11) and 12,200 r.p.m. (with the Microfuge 12) can be reached in 12 seconds. Protein precipitates, large particles, cell debris or cells can be precipitated from small volumes. The rotor can also be used for binding studies, serum separations and for separating organic-aqueous extraction phases.

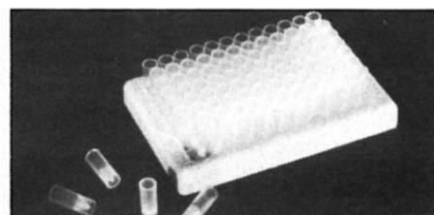
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● A new capillary blood collection system has been released by Sarstedt. Featuring an enlarged opening on the collector and new closure, this system is designed for collections of capillary blood of up to 1.0 ml. The system is available with a full range of anticoagulants and UV screening for the collection of UV sensitive analytes such as bilirubin.

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● B-J Scientific Products has recently added molybdenum and tantalum products to their crucible line, for use with strong mineral and organic acids and salts, and in the handling of molten metals. Both materials are resistant to acids, and tantalum also resists molten metals. Tantalum molybdenum crucibles are available in sizes from 5 ml to 1,000 ml, with or without covers.

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The RIA vial from Sarstedt



B J Scientific's new crucibles

● A new line of screw cap and septum closure type cuvettes, called the Capcell, has been introduced by TCS Medical Products. The Capcell line is available in pyrex and quartz for use in anaerobic and gas exchange measurements. The septa are Teflon-lined for complete inertness with volatile or corrosive liquids. Both cell types are available with all four sides polished for use in fluorescence studies or nephelometric measurements.

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● Reagent reservoirs moulded of polypropylene are now available from Robbins Scientific for use with multi-nozzle pipettors. The reservoirs can be used with volumes of between 1 and 75 ml. The reservoirs are autoclavable and are available either bulk-packed, or individually wrapped and sterilized by cobalt radiation.

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● J. Bibby Science Products have introduced a snap-on system for holding Quick-fit jointed glassware together, called Joint Clips. They will support a litre flask at temperatures up to 50° ambient or 80°C through the joint, reducing the need for clamping. Joint Clips are available in four sizes to match international conical joint sizes — 14/23, 19/26, 24/29 and 29/32.

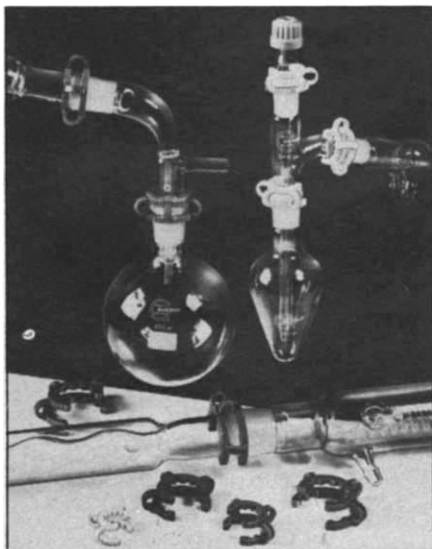
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● New from Denly Instruments is the Saitron model 311 digital thermometer. This is a two-digit microprobe thermometer, reading from 20°C to 40°C with an accuracy and precision of $\pm 0.03^\circ\text{C}$.

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● A new method of dealing with blood or urine spills is now available from Guest Medical in the form of Biozorb. It is an absorbent powder that contains calcium hypochlorite which has been demonstrated to be an effective inactivator of hepatitis B virus as well as other pathogens.

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Joint Clips from J Bibby Science Products

● The Aquavac from Uniscience is a new constant vacuum source. The two and four inlet port units of the Aquavac generate a constant water pressure which is independent of the mains water pressure. The system uses 10% of the water volume needed by water jet pumps. The water is recirculated so that only a small flow of water is required to maintain the continuous removal of condensed products.

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● The Harris Lo Temp 12 V upright freezer has a 12 cubic foot capacity and is divided into five chambers. Each chamber is fitted with a magnetically-attaching sub door. The Harris 12 V comes in three models: either -40°, -75° or -85°C low temperature point. Standard features on all models are the casters and handle, especially designed for safety. Options include air or water cooling, non-flammable refrigerant and stand-by CO₂ system to protect products from power or mechanical failure. The freezer is designed for work in the life sciences.

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● A new direct-reading density meter has been introduced by Paar Scientific. The Paar density meter operates over the range 15 to 50°C. The instrument provides information on the density, specific gravity or concentration of a liquid with a precision of 10^{-4} g per cm³. It needs no water bath since it has a built-in Peltier effect thermostat.

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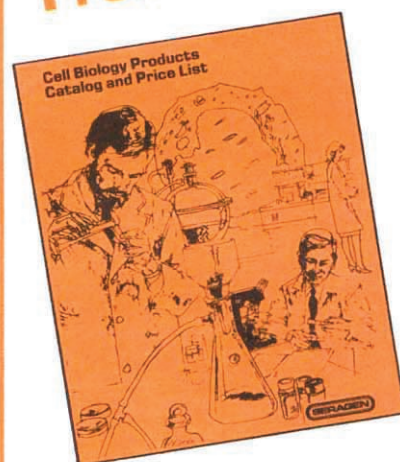
● Enzo Biochem now offers a new feature to the Enzo Bio-Probe system — a non-radioactive end-labelling kit. The specific DNA probe is first cut with DNase to yield fragments of a few hundred nucleotides and then end-labelled with biotinylated dUTP catalysed by terminal deoxynucleotide transferase (TdT). This results in an increase in DNA labelling with a corresponding increase in detection sensitivity. TdT biotinylated probes are stable for at least one year at 4°C and hybridize normally to homologous target DNA. Detection of the hybridized biotinylated DNA probe can be carried out on nitrocellulose membranes using Detek I-h.r.p. (horseradish peroxidase), an enzymatic colourimetric detection system. Detek I-h.r.p. consists of a streptavidin and biotinylated horseradish peroxidase and is visualized using standard horseradish peroxidase substrates.

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● New cellulose nitrate (nitrocellulose) membrane sheets are now available from Whatman for blot transfer and binding applications. The new sheets are designed for nucleic acid and protein binding studies in molecular biology and genetic engineering. The new membrane sheet material is the same as that used in Whatman's cellulose nitrate membrane filters (Type WCN).

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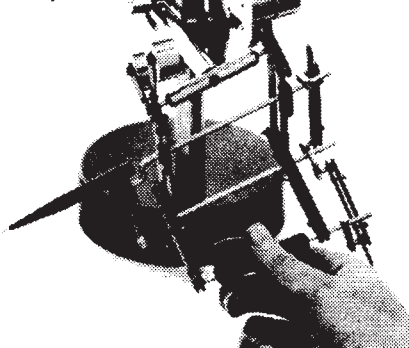
Separating techniques

● SynChropak 0300 is an anion-exchange support for HPLC and is produced by Syn-Chrom. The medium consists of a macroporous silica base of 300 Å, although other pore sizes, such as 1,000 Å are also available.

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● For removing endoproteases from biological fluids, Boehringer Mannheim has developed a new "sandwich affinity chromatography" method with α_2 -macroglobulin bound to a zinc chelate carrier. The affinity matrix "carrier-bound α_2 -macroglobulin" will remove almost all endoproteases from solutions such as crude extracts, enzyme solutions or cell culture media. Trypsin, chymotrypsin, elastase, papain, bromelain, ficin, dispase and thermolysine have been shown to be absorbed. However, a few specific proteases such as endoproteinase Lys-C and collagenase from *Clostridium histolyticum* and exoproteases such as carboxypeptidase A and Y are not bound. The material is suitable for column and batch processes. The binding capacity is around 13 nmol of proteinase per ml of column volume.

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● The Dionex series 2000i ion chromatographs will perform amino acid analysis, analyse fermentation broths and detect carbohydrates. The new 2011i chromatographs now incorporate an IonChrom Pulsed Amperometric Detector which allows large polysaccharides to be separated and detected. In fermentation broths, anions, cations, organic acids and transition metals can now be detected.

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● The Biochemistry Group at Harwell has developed a continuous electrophoretic separator for isolating and purifying biochemical materials on an industrial scale. It is to be manufactured and sold worldwide by CJB Developments. The separator allows large scale continuous electrophoretic separation of mixtures in free solution. The machine will process 2.4 litres of mixture per hour and up to 29 separate fractions can be drawn off simultaneously. It operates under mild mechanical, chemical and thermal conditions, enabling sensitive materials to be processed with little loss of activity. One particular application is in the preparation of anti-haemophilic blood clotting Factor VIII. It uses a rotary system to stabilize the laminar flow of a carrier solution into which the migrant is injected. This laminar flow is maintained as the particles or solutes of different charge migrate towards the cathode or anode, and allows the separated fractions to be drawn off.

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● The latest Fotodyne product guide for researchers specializing in DNA research, thin layer chromatography, electrophoresis and scientific and medical photographic documentation is now available. The Fotodyne product guide offers DNA transilluminators, UV blocking face shields, high performance radial chromatography, DNA power supplies and CRT imaging.

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● Millipore has developed a versatile, rapid filtration method that can be applied directly to a fermenter to reduce volumes by up to 100 times and to wash cells without centrifugation. The Millipore Pellicon ultrafiltration system can provide the ability to increase parameters of rate, recovery, resolution and load capacity for harvesting cell mass from fermentation and concentrating both extracellular and intracellular soluble products. All operations are performed in a closed system. No potentially hazardous aerosols are generated, and the system is virtually silent. The system maintains cell viability and can be sterilized with water.

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● Beckman Instruments has introduced the Spherogel TSK-IEX preparative column to allow chemists to scale up ion-exchange separations in biotechnology, biomedical research and industrial research applications. The preparative column measures 21.5 mm x 150 mm. It comes in DEAE and CM forms to enable anionic and cationic preparative separations. The chemistries are identical to that of the Spherogel TSK-IEX analytical columns.

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● A new version of their magnetic filter funnel is now available from Gelman Sciences. The 47 mm funnel is designed for the vacuum filtration of liquids and is suited to analysis of microbiological and particulate contamination using the membrane filtration technique. Any 47 mm filter membrane can be used in the funnel, which holds 300 ml. The unit can be washed and autoclaved and carries a six-month guarantee. It can be operated with one hand and has a dual magnet seal to assure that no leakage occurs. A ridge on the base of the stem guides and holds the filter membrane in position, while forceps access points provide easy retrieval of the filter.

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The magnetic filter funnel from Gelman